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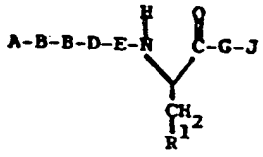
(54) Peptide enzyme inhibitors.

(57) Enzyme peptides of the formula



(I.)

and analogs thereof which inhibit renin and are useful for treating various forms of renin-associated hypertension and hyperaldosteronism.

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Renin is a proteolytic enzyme of molecular weight about 40,000, produced and secreted by the juxtaglomerular cells of the kidney. Renin acts on the plasma substrate, angiotensinogen, to split off the decapeptide angiotensin I, which is converted to the potent pressor agent angiotensin II. Thus, the renin-angiotensin system plays an important role in normal cardiovascular homeostasis and in some forms of hypertension.

In the past, attempts to modulate or manipulate the renin-angiotensin system have met with success in the use of inhibitors of angiotensin I converting enzyme. In view of this success, it was concluded that a specific inhibitor of the limiting enzymatic step that ultimately regulates angiotensin II production, the action of renin on its substrate, would be at least equally successful. Thus, an effective inhibitor of renin has been long sought as both a therapeutic agent and as an investigative tool.

2. Brief Description of the Prior Art

There has been substantial interest in the synthesis of useful renin inhibitors for many decades; and the following table lists the major classes of renin inhibitors that have been studied, as well as their relative inhibition constants (K_i):

Class	K_i (M)
Renin antibody	probably 10^{-6}
Pepstatin	$10^{-6} - 10^{-7}$
Phospholipids	10^{-3}
Substrat analogs	
Tetrapeptides	10^{-3}
Octa- to tridecapeptides	$10^{-5} - 10^{-6}$

renin substrate, human renin, on the other hand, does cleave the pig renin substrate. See Poulsen et al., Biochim. Biophys. Acta 452:533-537, 1976; and Skeggs, Jr. et al., J. Exp. Med. 106:439-453, 1957. Moreover, the human renin inhibitory activity of the peptides of the present invention most active in inhibiting pig renin has been confirmed, thus providing further evidence of this accepted correlation between human and pig renin activity.

It has been found, for example, using pig renin substrate analogy, that the octapeptide sequence extending from histidine-6 through tyrosine-13 has kinetic parameters essentially the same as those of the full tetradecapeptide renin substrate. The amino acid sequence of the octapeptide in pig renin substrate is as follows:

6 7 8 9 10 11 12 13
-His-Pro-Phe-His-Leu-Leu-Val-Tyr-

Renin cleaves this substrate between Leu^{10} and Leu^{11}.

Kokubu et al., Biochem. Pharmacol. 22: 3217-3223, 1973, synthesized a number of analogs of the tetrapeptide found between residues 10 to 13, but while inhibition could be shown, inhibitory constants were only of the order of 10^{-3}M.

Analogs of a larger segment of renin substrate were also synthesized: Burton et al., Biochemistry 14: 3892-3898, 1975, and Poulsen et al., Biochemistry 12: 3877-3882, 1973. Two of the major obstacles which had to be overcome to obtain an effective renin inhibitor useful in vivo were lack of

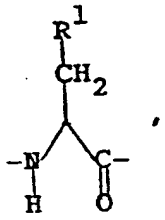
state for pepsin hydrolysis of peptide bonds. However, the applicability of these concepts to renin inhibitors is not taught in any of these references, and would be speculative due to the known high degree of specificity of the renin enzyme.

Kokubu et al., Biochem. Biophys. Res. Comm. 118:929-933, 1984; and Fehrentz et al., FEBS Letters 167: 273-276, 1984, have prepared a renin inhibitor in which a C-terminal aldehyde is used to mimic Leu^{10} of the substrate. However, there is no suggestion of the renin inhibitors of the present invention in which statine and other moieties replace Leu^{10}-Leu^{11} of the substrate.

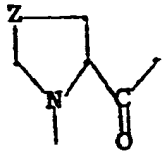
Veber and Rich, in U.S. Patent No. 4,384,994 and published European Patent Application No. 0,077,029; Evans and Rittle, in U.S. Patent No. 4,397,786; Veber and Boger, in published European Patent Application No. 0,077,028; Boger et al, Nature, 303:81-84 (1983); have all described renin inhibitory peptides containing statine; and in Nature there is further described renin inhibitors having a shortened C-terminus, with a non-peptide ending after the 11-position. However, none of these references describe or suggest the renin inhibitors of the present invention and the significant increase in renin inhibitory activity obtainable therewith. Moreover, the Nature reference teaches away from renin inhibitors having non-peptide components after the 11-position, as with the inhibitors of the present invention.

For other articles describing previous efforts to devise renin inhibitors, see Marshall, Federation Proc. 35: 2494-2501, 1976; Burton et al.,

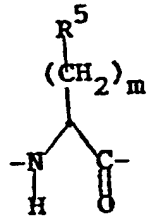
substituted with up to five members independently selected from the group consisting of C_{1-8}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, and halo; n is 0 to 5; m is 0 to 2; and p is 0 to 2; except that where X is -O-, only one of R^2_a or R^2_b is present;

B is absent; glycyl; sarcosyl; or  ,

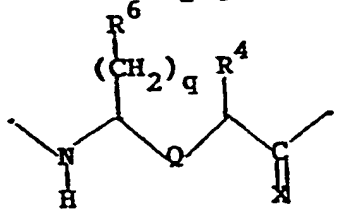
where R^1 is as defined further below;

D is absent; or  , where Z is

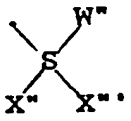
$-(CH_2)_1-$ and 1 is 1 or 2; or -S-;

E is absent; or  , where m is 1 to 4; and

R^5 is hydrogen; C_{1-4} alkyl; aryl; aryl-C_{1-4} alkyl; aryl C_{1-4} alkyl or aryl where the aryl portion is substituted with up to three members selected from the group consisting of C_{1-4} alkyl, trifluoromethyl, hydroxy, C_{1-4} alkoxy, and halo; or indolyl;

G is (1)  , where q is 1 to 4; X is O, or H, H;

wherein X' is hydroxy; OR_4' wherein R_4' is as defined below; amino; or mono- or di-C_{1-4}alkyl amino; and W' is absent; -O-; -NH-; or $-CH_2-$;



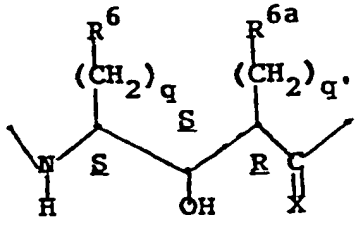
where X" and X"' are independently

absent; or  ; and

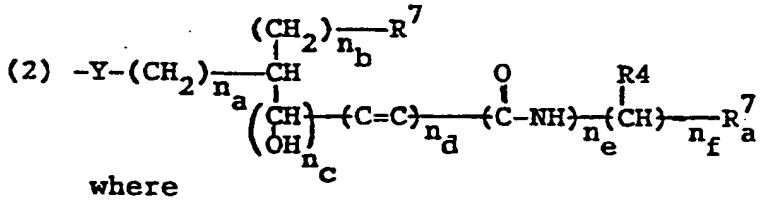
W" is absent; $-CH_2-$; or  , where R^8 is hydrogen or C_{1-3} alkyl;

 ;  ; or

 ; where R is hydrogen; C_{1-4} alkyl; formyl; C_{1-4} alkanoyl; aroyl; carboxy; C_{1-4} alkoxycarbonyl; aryl-oxycarbonyl; or aryl C_{1-4} alkoxycarbonyl; or

(2)  where q is 1 to 4; q' is 0 to 4; X is O or H, H;

heterocyclic C_{1-4}alkyl; $N(R')^+_3A^-$, where R' is as defined above, and A^- is a counterion; guanidyl; heterocyclic; heterocyclic substituted with up to five members independently selected from the group consisting of C_{1-6}alkyl, hydroxy, trifluoromethyl, C_{1-4}alkoxy, halo, aryl, aryl C_{1-4}alkyl, amino, and mono- or di-C_{1-4}alkylamino; or heterocyclic substituted with another, the same or different, heterocyclic;

(2) 

where

Y is as defined above;

n_a is 0 or 1;

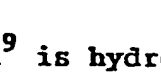
n_b is 1 to 4;

n_c is 0 or 1;

n_d is 0 or 1;

n_e is 0 or 1, provided that n_e cannot be 1 when n_d is 0;

n_f is 1 to 4;

R^4 is hydrogen; or  , where

R^9 is hydrogen; C_{1-4}alkyl; hydroxy; or C_{3-7}cycloalkyl; and R^3 is hydrogen; C_{1-4}alkyl; aryl;

(b) $-(CH_2)_n-\underset{\underset{CH_2}{|}}{C}-$

where

n is 0 or 1; or

(4) (a) $Y-\underset{\underset{q}{|}}{\overset{\overset{R^{10}}{|}}{(CH)}}-R^{11}$; (b) $Y-\underset{\underset{q'}{|}}{\overset{\overset{R^{12}}{|}}{(CH)}}-R^{13}$; or

(c) $Y-\underset{\underset{R^{14}}{|}}{CH}-R^{11}$

where

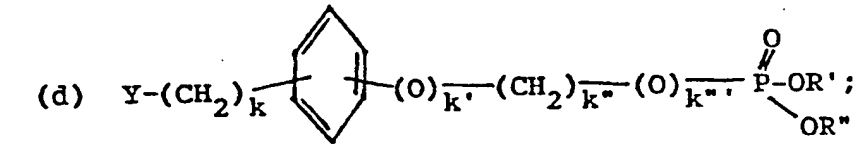
Y is -NH- or -O-;

q is 1-5;

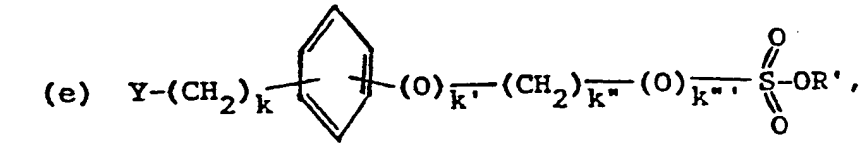
q' is 0-5;

R^{10} is hydrogen; hydroxy; $N(R'')_2$, where R'' may be the same or different and is hydrogen or C_{1-4}alkyl; guanidyl; or $N^{\oplus}(R'')_3A^{\ominus}$, where R'' is as defined above, and $A^{\ominus}$ is a counterion; provided that at least one R^{10} is not hydrogen;

R^{11} is C_{1-4}alkyl; C_{3-7}cycloalkyl; aryl; aryl substituted with up to three members independently selected from the group consisting of C_{1-6}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, amino, mono- or di- C_{1-4}alkylamino, amino C_{1-4}alkyl, mono-, di-, or tri-C_{1-4}alkylamino-C_{1-4}alkyl, halo, carboxy, carboxy ester or amide, carboxy-C_{1-4}-alkoxy, carboxy-C_{1-4}-alkoxy ester or amide, α-aminocarboxy-

(d) 

or

(e) 

where

Y is -NH- or -O-;

k is 0-4;

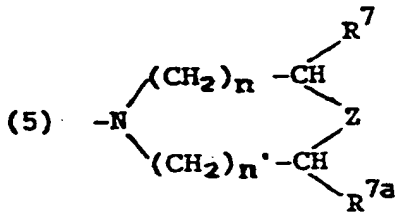
k' is 0 or 1;

k" is 0-4;

k"'is 0 or 1;

R' is hydrogen or C_{1-4}alkyl; and

R" is hydrogen or C_{1-4}alkyl;

(5) 

where Z is NH, N-R^7,O,S or CHR^7;

n' is 0 to 5; and

R^{7a} is hydrogen, hydroxy, C_{1-4}-alkyl,C_{3-7}-cydoalkyl, aryl,ary 1 substituted with from one to five members independently selected from the group consisting of C_{1-6}-alkyl trifluoromethyl, hydroxy, C_{1-4}alkoxy, amino, mono- or di- C_{1-4} alkylamino, and halo; $N(R')_2$, where R' may be the same or different and is hydrogen,

included in the peptides of the present invention, preferred chiralities are indicated in the description which follows.

In the above definitions, the term "alkyl" is intended to include both branched and straight chain hydrocarbon groups having the indicated number of carbon atoms.

The term "halo" means fluoro, chloro, bromo and iodo.

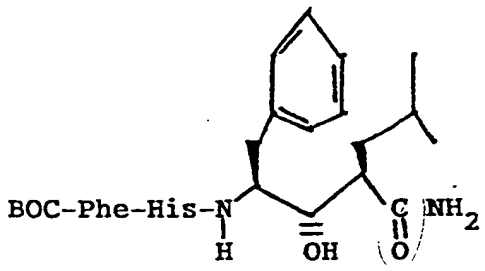
The aryl substituent represents phenyl, and naphthyl.

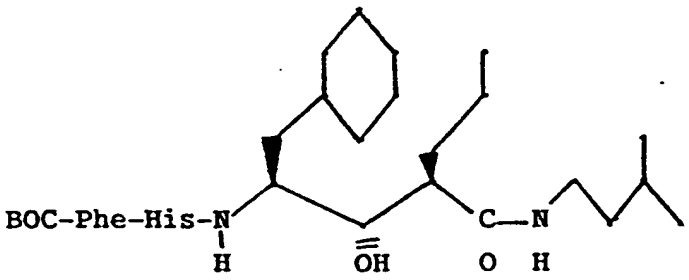
The heterocyclic substituent recited above represents any 5- or 6-membered ring containing from one to three heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur; having various degrees of unsaturation; wherein the nitrogen and sulfur heteroatoms may optionally be oxidized; wherein the nitrogen heteroatom may optionally be quaternized; and including any bicyclic group in which any of the above heterocyclic rings is fused to a benzene ring. Heterocyclic substituents in which nitrogen is the heteroatom are preferred, and of these, those containing a single nitrogen atom are preferred. Fully saturated heterocyclic substituents are also preferred. Thus, piperidine is a preferred heterocyclic substituent. Other preferred heterocyclic substituents are: pyrryl, pyrrolinyl, pyrrolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyridyl, piperidinyl, pyrazinyl, piperazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, indolyl, quinolinyl,

It will be understood that closely related analogs of the above common amino acids, for example, aliphatic amino acids in addition to Ala, Val, Leu, and Ile, such as α-aminobutyric acid (Abu), and substituted phenyl derivatives of Phe, are included in the broad description of the novel inhibitory peptides of the present invention represented by Formula I and its definitions. Thus, the peptides of Formula II and its definitions represent preferred peptides of the present invention.

Preferred inhibitory peptides of the present invention are the following:

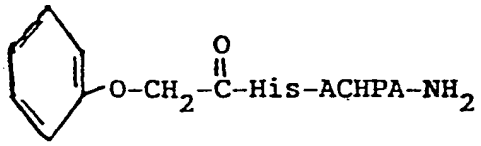
BOC^1-His-Pro-Phe-His-Sta-OEt

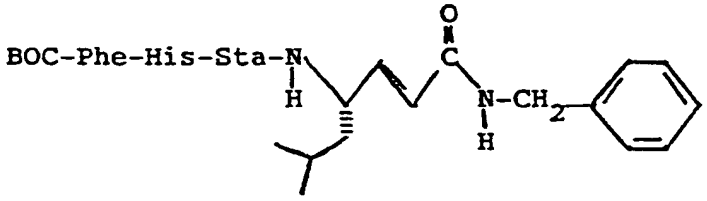


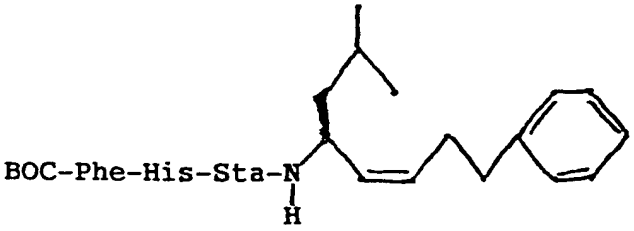


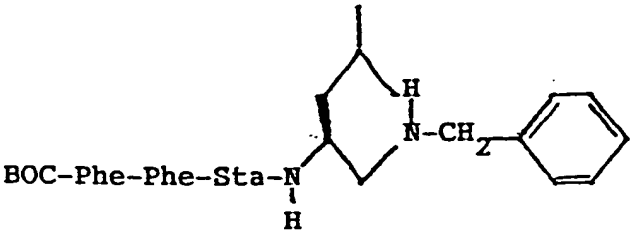
$BOC-Phe-His-ACHPA^2-NH_2$

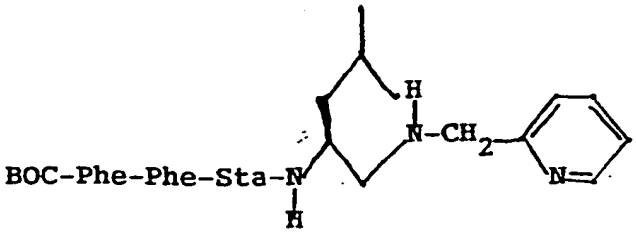
$BOC-HomoPhe-His-Sta-NH_2$

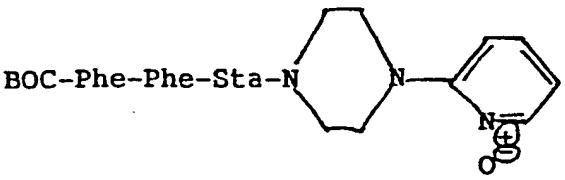


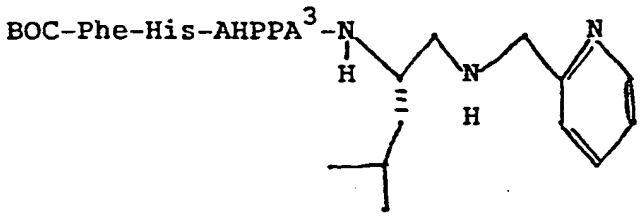


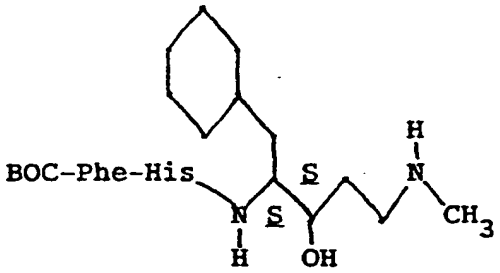


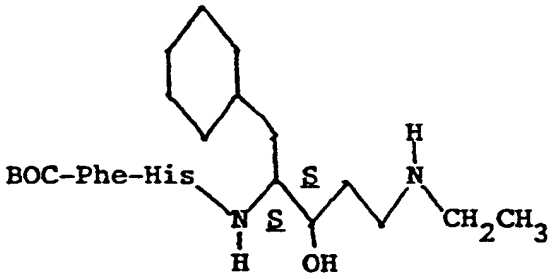


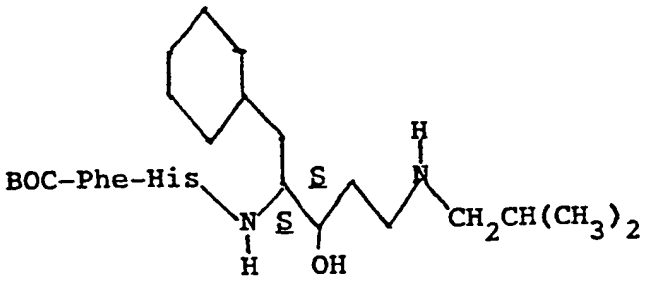


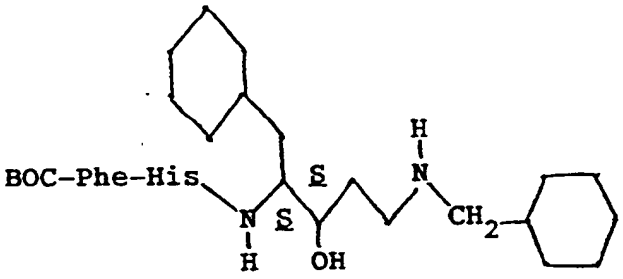

BOC-Phe-Phe-Sta-N
N
N
O

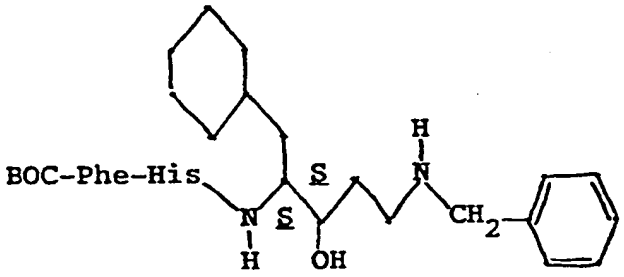

BOC-Phe-His-AHPPA
3
-N
H
N
H
N

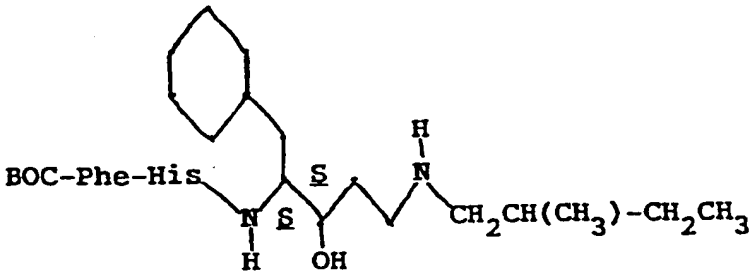

BOC-Phe-His
S
N
H
S
OH
H
N
CH3

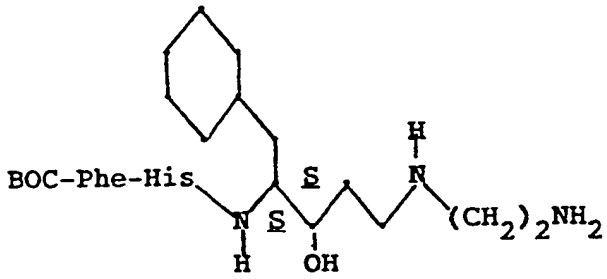

BOC-Phe-His
S
N
H
S
OH
H
N
CH2CH3

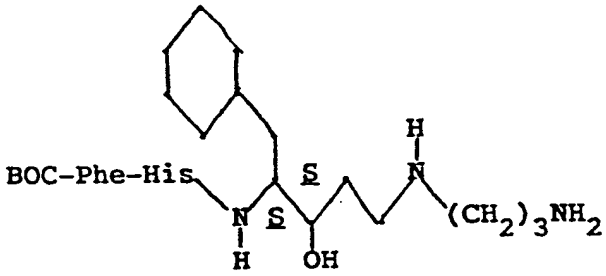

BOC-Phe-His
S
S
H
N
N
H
OH
CH2CH(CH3)2

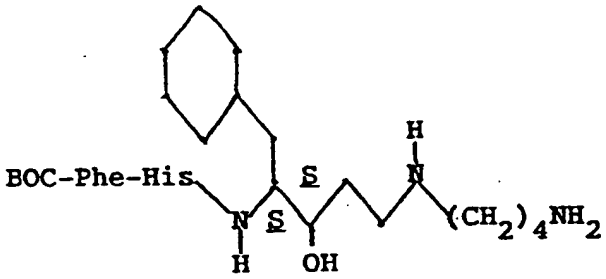

BOC-Phe-His
S
S
H
N
N
H
OH
CH2

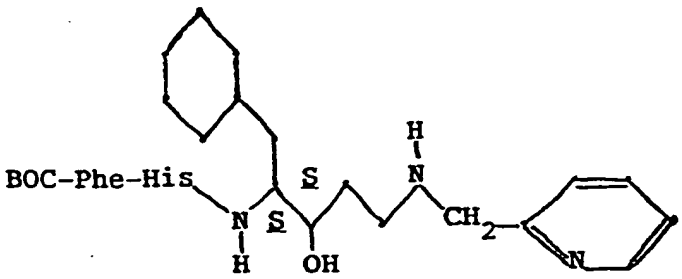

BOC-Phe-His
S
S
H
N
N
H
OH
CH2

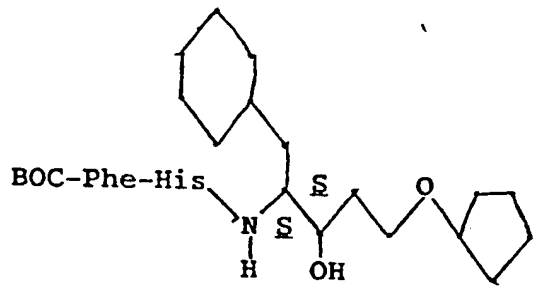

BOC-Phe-His
S
S
H
N
N
H
OH
CH2CH(CH3)-CH2CH3

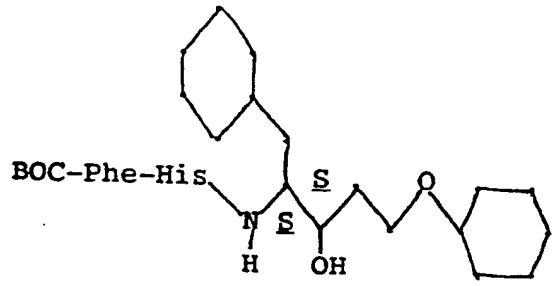

BOC-Phe-His
S
H
N
S
N
H
OH
$(CH_2)_2NH_2$

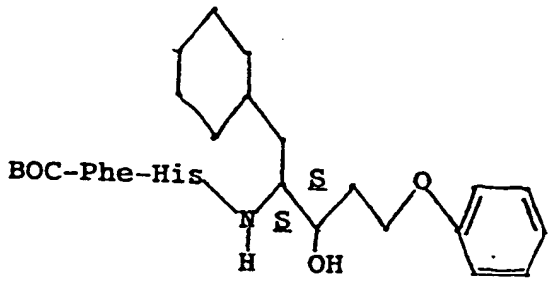

BOC-Phe-His
S
H
N
S
N
H
OH
$(CH_2)_3NH_2$

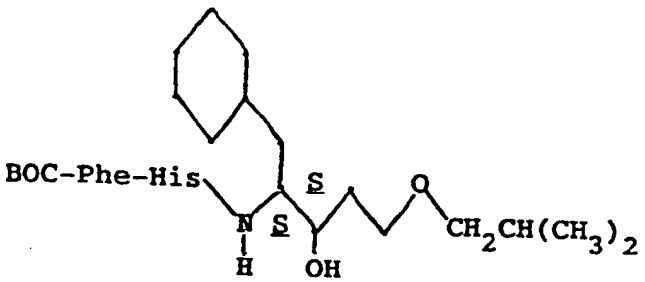

BOC-Phe-His
S
H
N
S
N
H
OH
$(CH_2)_4NH_2$

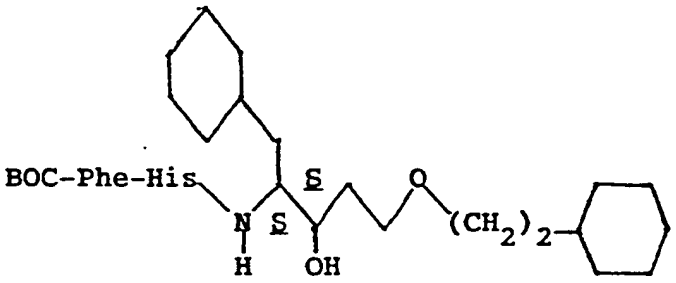

BOC-Phe-His
S
H
N
S
N
H
OH
CH_2
N

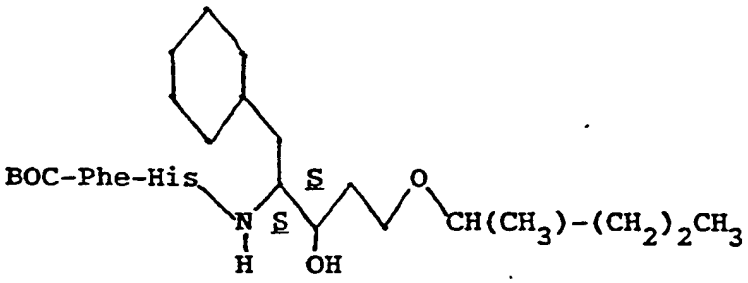

BOC-Phe-His
S
N
S
H
OH
O

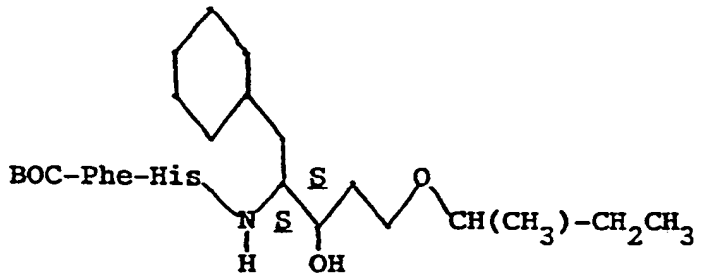

BOC-Phe-His
S
N
S
H
OH
O

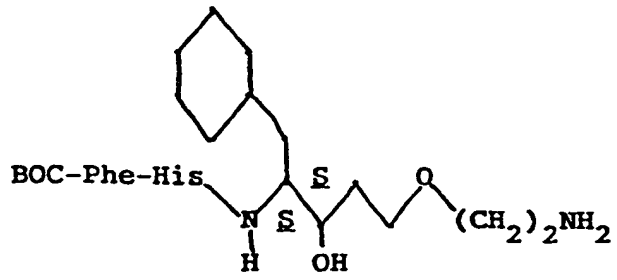

BOC-Phe-His
S
N
S
H
OH
O

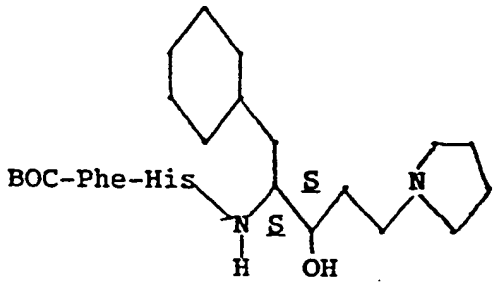

BOC-Phe-His
S
N
S
H
OH
O
CH2CH(CH3)2

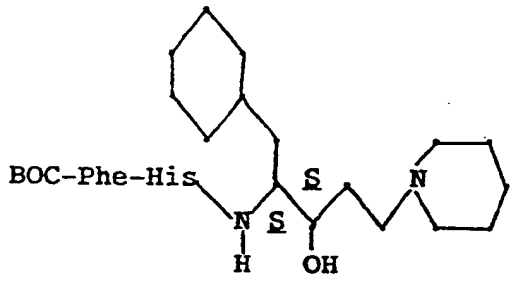

BOC-Phe-His
S
N
S
H
OH
O
(CH2)2

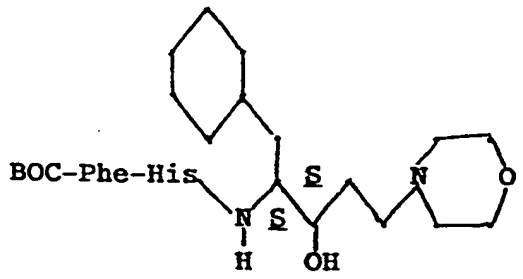

BOC-Phe-His
S
N
S
H
OH
O
CH(CH3)-(CH2)2CH3

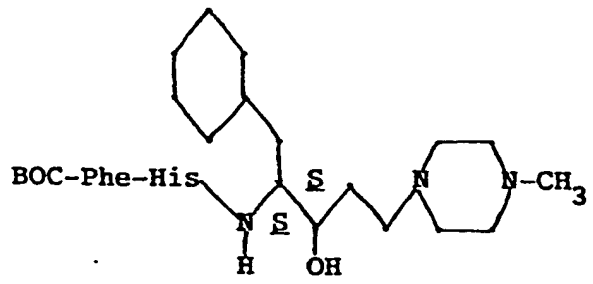

BOC-Phe-His
S
N
S
H
OH
O
CH(CH3)-CH2CH3

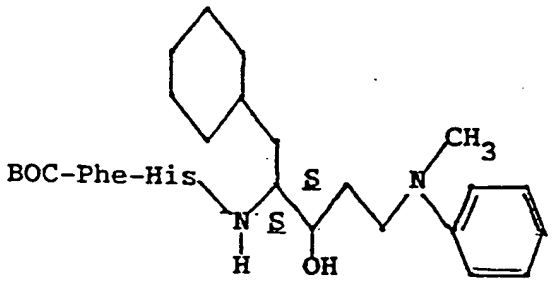

BOC-Phe-His
S
N
S
H
OH
O
(CH2)2NH2

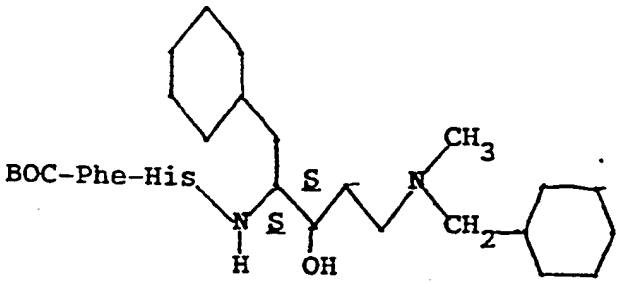

BOC-Phe-His
S
N
S
N
H
OH

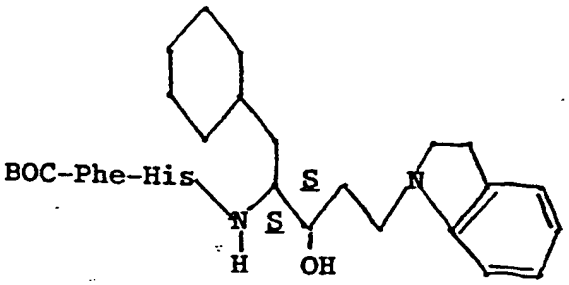

BOC-Phe-His
S
N
S
N
H
OH

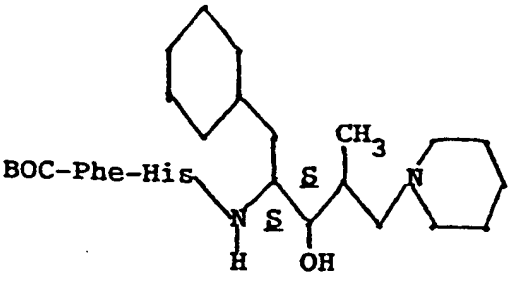

BOC-Phe-His
S
N
O
S
N
H
OH

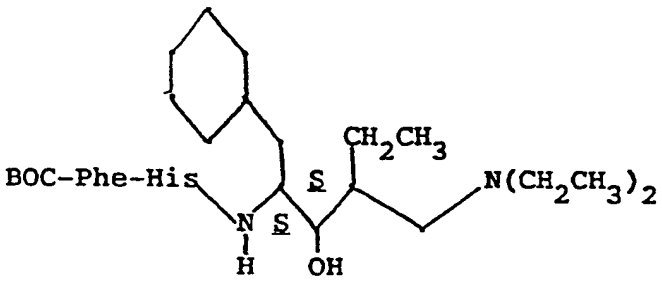

BOC-Phe-His
S
N
N-CH3
S
N
H
OH

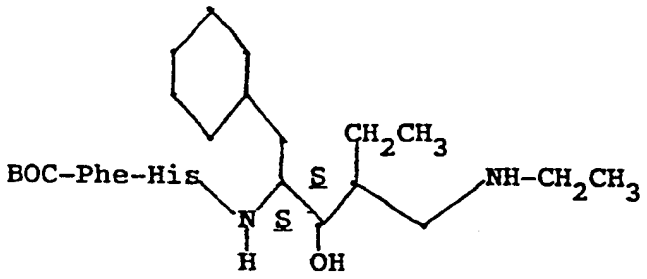

BOC-Phe-His
CH3
S
N
S
N
H
OH

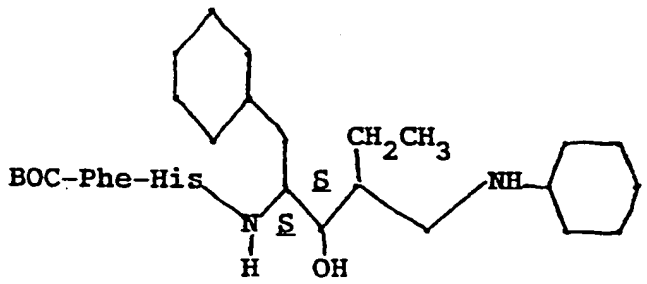

BOC-Phe-His
CH3
S
N
S
N
H
OH
CH2

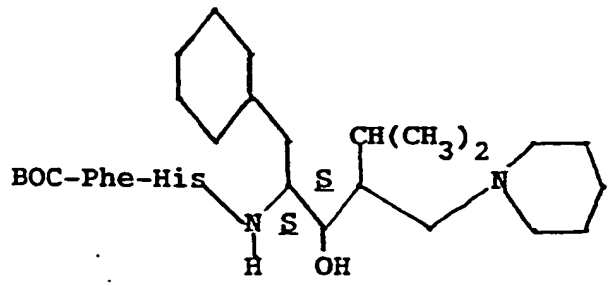

BOC-Phe-His
S
N
S
N
H
OH

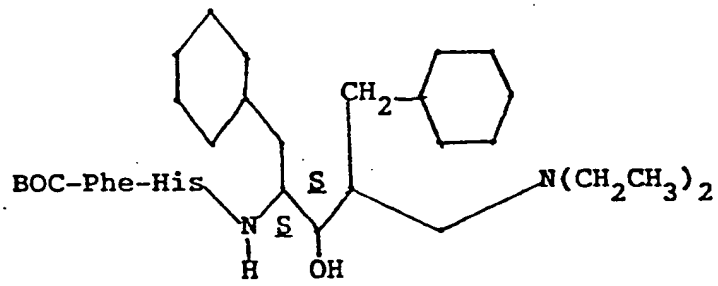

BOC-Phe-His
CH3
S
N
S
N
H
OH

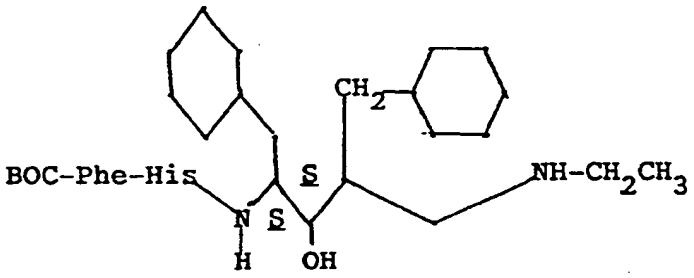

BOC-Phe-His
S
CH2CH3
N(CH2CH3)2
N
S
H
OH

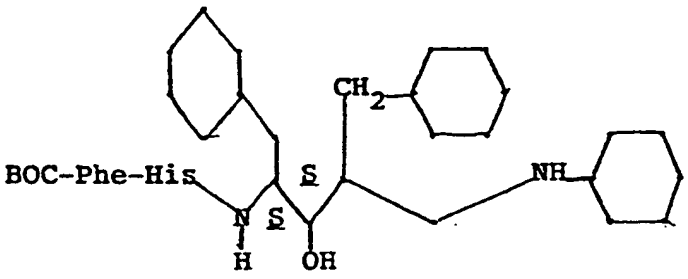

BOC-Phe-His
S
CH2CH3
NH-CH2CH3
N
S
H
OH

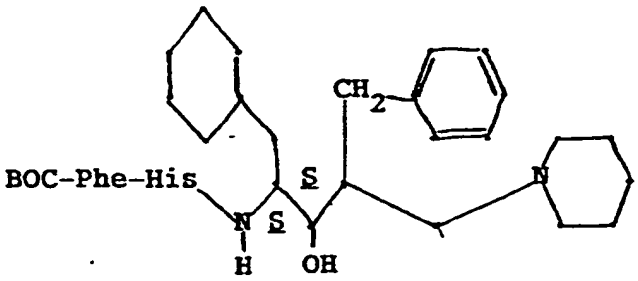

BOC-Phe-His
S
CH2CH3
NH
N
S
H
OH

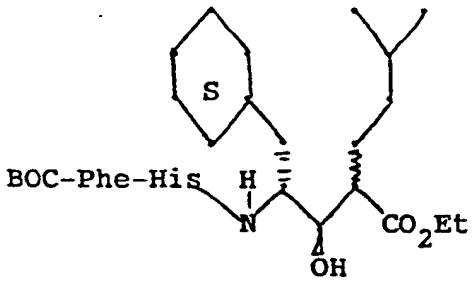

BOC-Phe-His
S
CH(CH3)2
N
N
S
H
OH

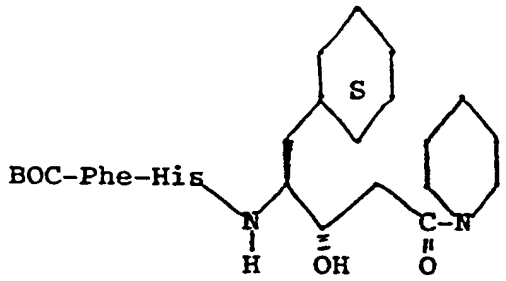

BOC-Phe-His
S
N
S
H
OH
CH2
N(CH2CH3)2

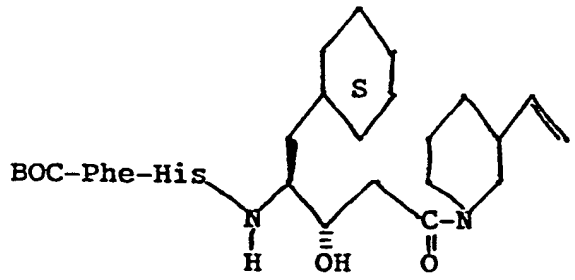

BOC-Phe-His
S
N
S
H
OH
CH2
NH-CH2CH3

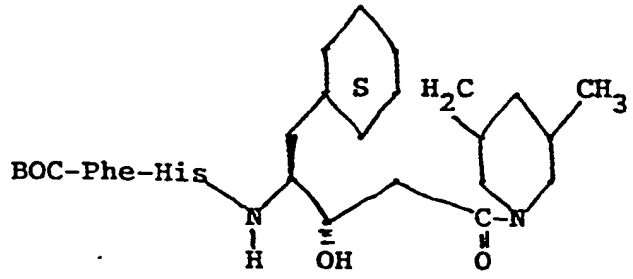

BOC-Phe-His
S
N
S
H
OH
CH2
NH

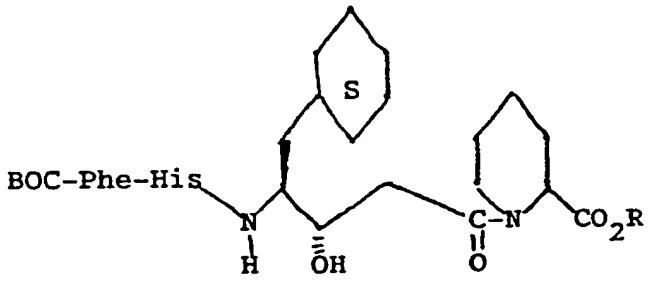

BOC-Phe-His
S
N
S
H
OH
CH2
N


S
BOC-Phe-His
H
N
OH
CO_2Et

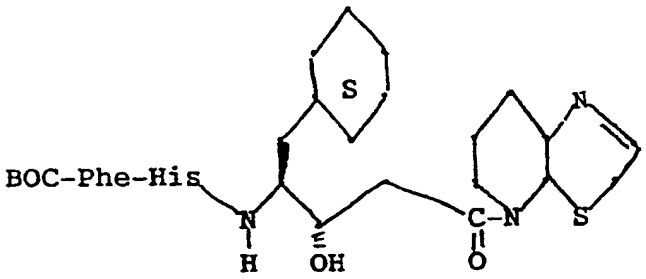

S
BOC-Phe-His
N
H
OH
C-N
O

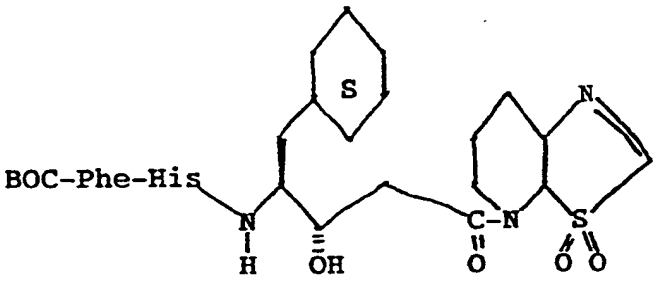

S
BOC-Phe-His
N
H
OH
C-N
O

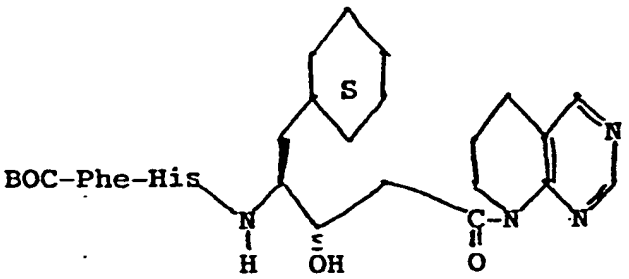

S
H_2C
CH_3
BOC-Phe-His
N
H
OH
C-N
O

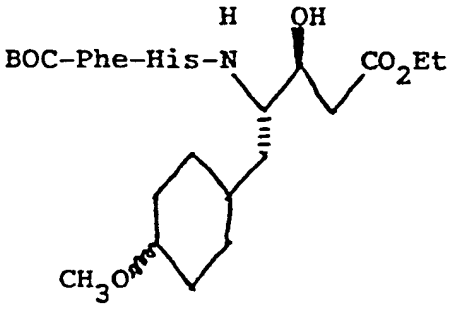


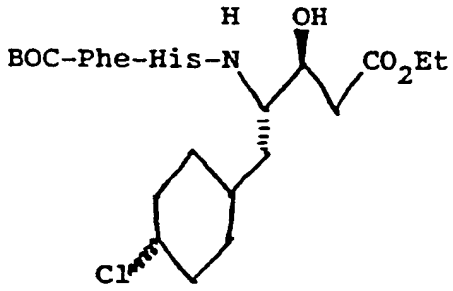
where R=alkyl, aryl, CH_2OH

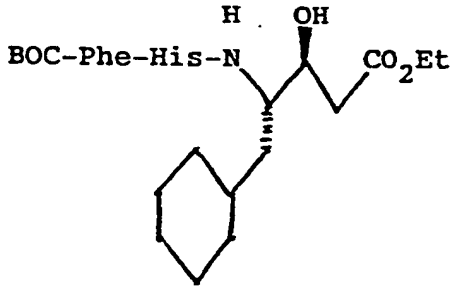


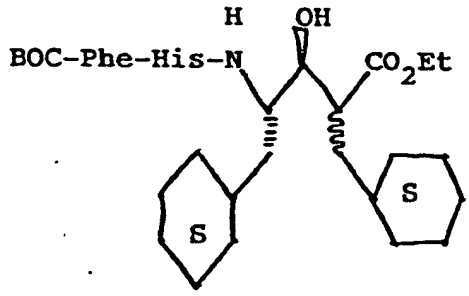


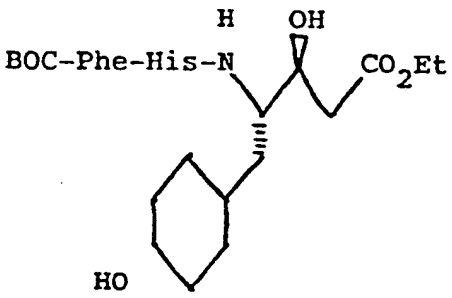


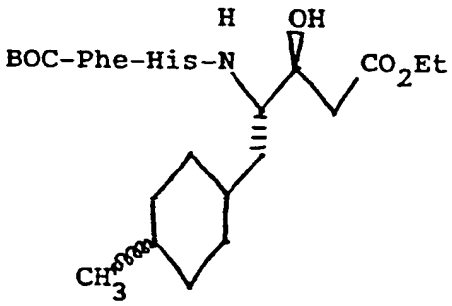

H
OH
BOC-Phe-His-N
CO2Et
CH3O

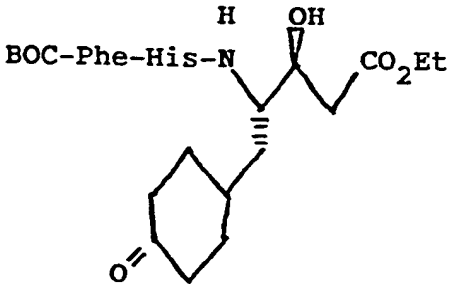

H
OH
BOC-Phe-His-N
CO2Et
Cl

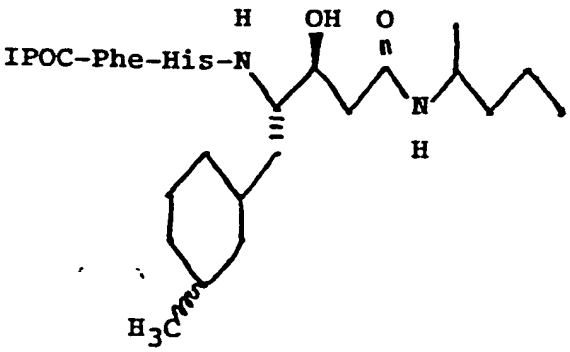

H
OH
BOC-Phe-His-N
CO2Et

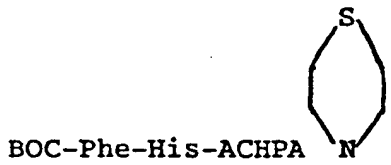

H
OH
BOC-Phe-His-N
CO2Et
S
S


H
OH
BOC-Phe-His-N
CO2Et
HO


H
OH
BOC-Phe-His-N
CO2Et
CH3


H
OH
BOC-Phe-His-N
CO2Et
O

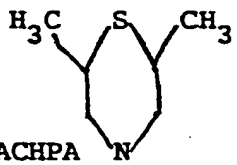

H
OH
O
IPOC-Phe-His-N
N
H
H3C



BOC-Phe-His-ACHPA

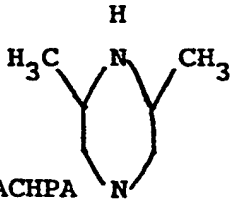
BOC-Phe-His-ACHPA

BOC-Phe-His-ACHPA

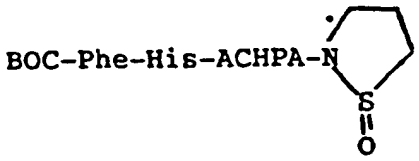


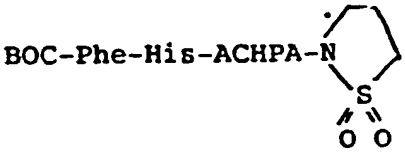
BOC-Phe-His-ACHPA

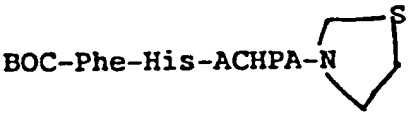
BOC-Phe-His-ACHPA

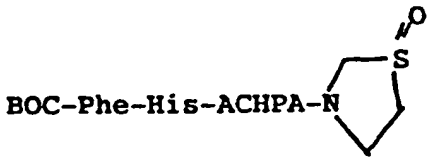


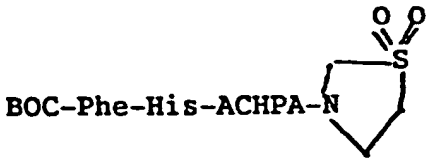
BOC-Phe-His-ACHPA


BOC-Phe-His-ACHPA-N
S
O


BOC-Phe-His-ACHPA-N
S
O O


BOC-Phe-His-ACHPA-N
S

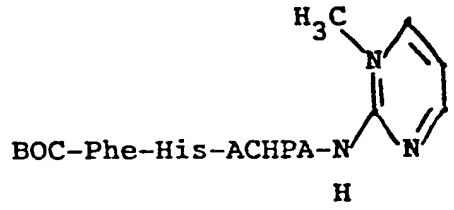

BOC-Phe-His-ACHPA-N
O
S

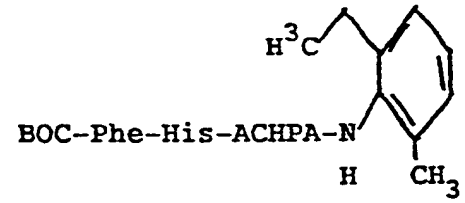

BOC-Phe-His-ACHPA-N
O O
S

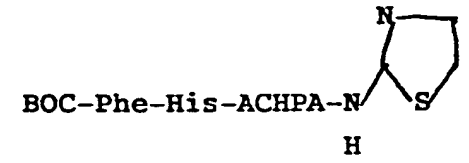

BOC-Phe-His-ACHPA-N
N-CH2

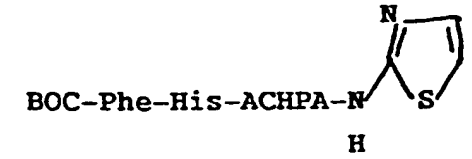

BOC-Ph -His-ACHPA-N
O

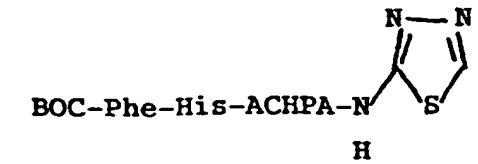

BOC-Phe-His-ACHPA-N
H
N
N

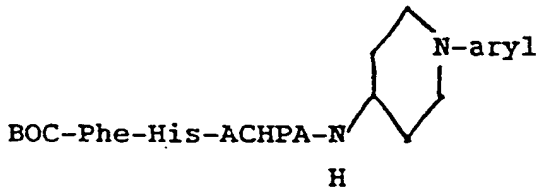

BOC-Phe-His-ACHPA-N
H
H3C
N
N

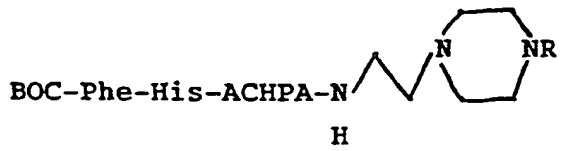

BOC-Phe-His-ACHPA-N
H
H3C
CH3

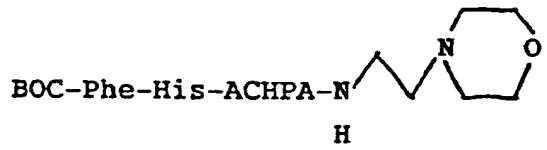

BOC-Phe-His-ACHPA-N
H
N
S

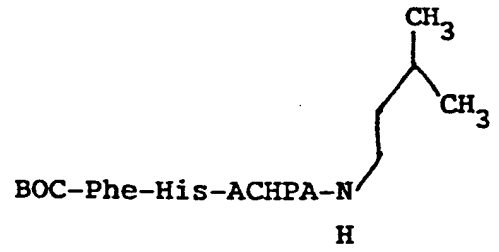

BOC-Phe-His-ACHPA-N
H
N
S

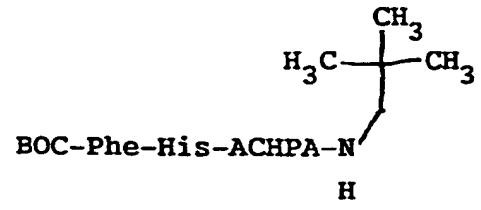

BOC-Phe-His-ACHPA-N
H
N
N
S

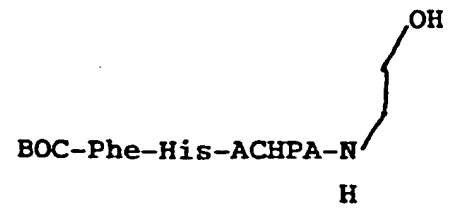

N-aryl
BOC-Phe-His-ACHPA-N
H

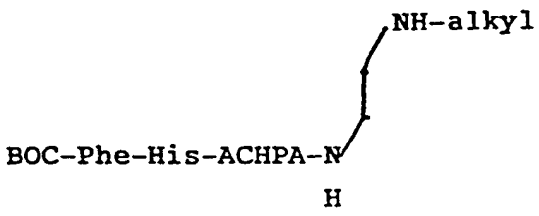

N
NR
where R=H, alkyl
BOC-Phe-His-ACHPA-N
H

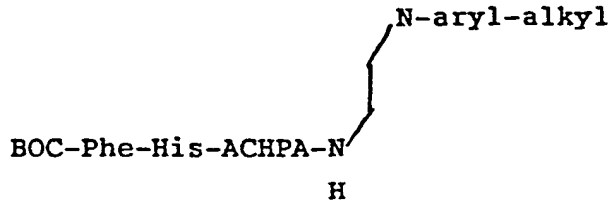

N
O
BOC-Phe-His-ACHPA-N
H

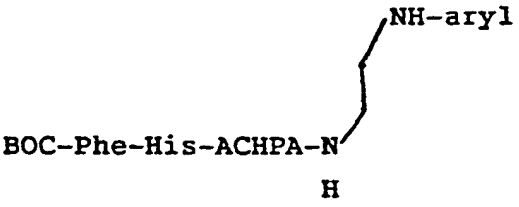

CH3
CH3
BOC-Phe-His-ACHPA-N
H


CH3
H3C
CH3
BOC-Phe-His-ACHPA-N
H


OH
BOC-Phe-His-ACHPA-N
H


NH-alkyl
BOC-Phe-His-ACHPA-N
H


N-aryl-alkyl
BOC-Phe-His-ACHPA-N
H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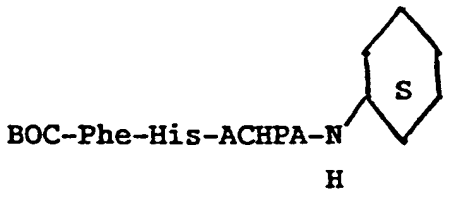
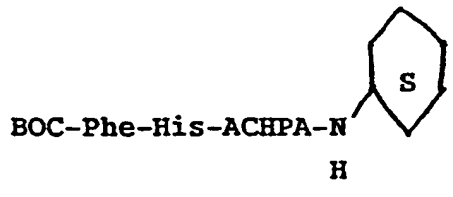
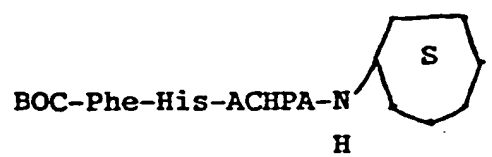
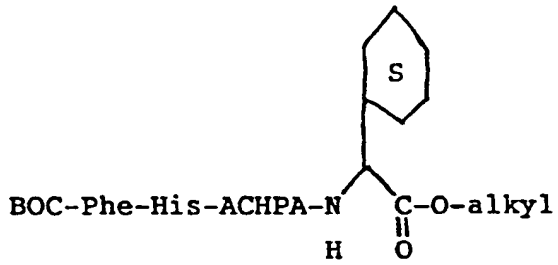
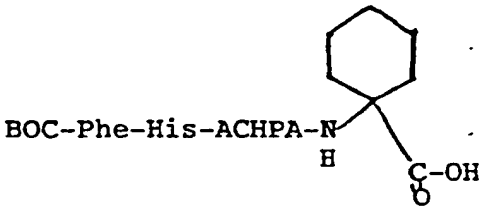
BOC-Phe-His-ACHPA-N
H

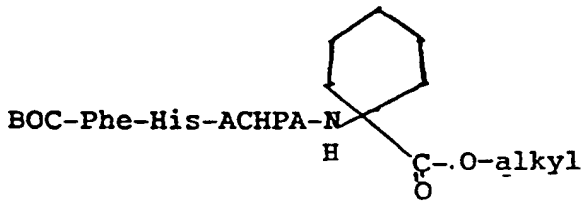

S
BOC-Phe-His-ACHPA-N
H

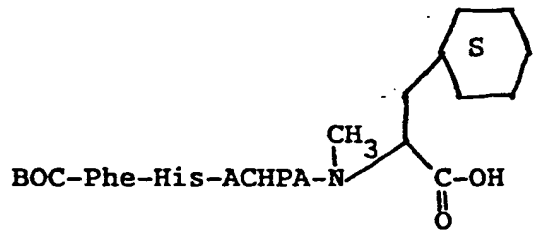

S
BOC-Phe-His-ACHPA-N
H

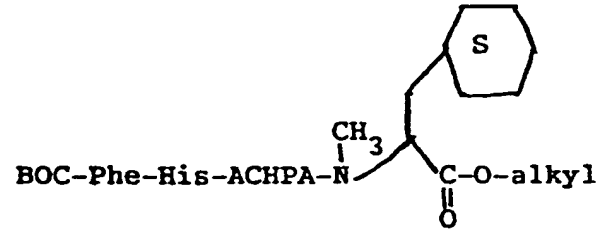

S
BOC-Phe-His-ACHPA-N
H

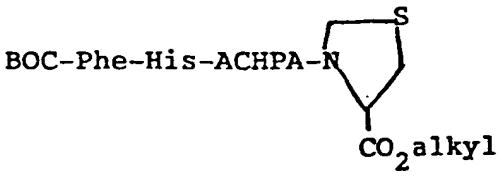

S
BOC-Phe-His-ACHPA-N
C-O-alkyl
H
O

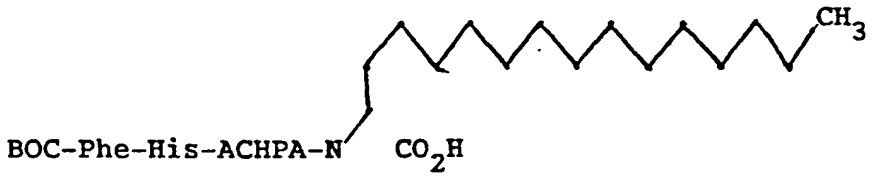

BOC-Phe-His-ACHPA-N
H
C-OH
O

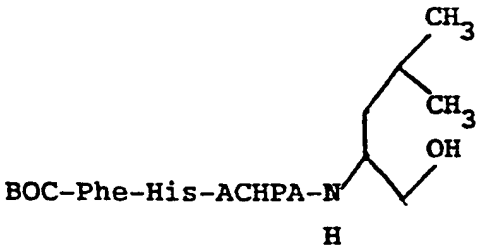

BOC-Phe-His-ACHPA-N
H
C-O-alkyl
O

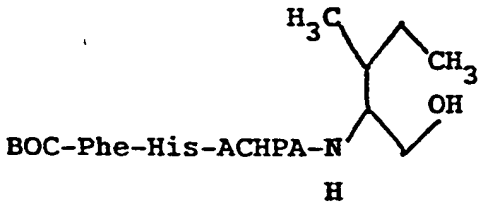

S
CH3
BOC-Phe-His-ACHPA-N
C-OH
O

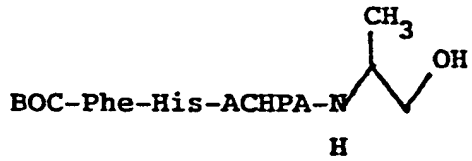

S
CH3
BOC-Phe-His-ACHPA-N
C-O-alkyl
O









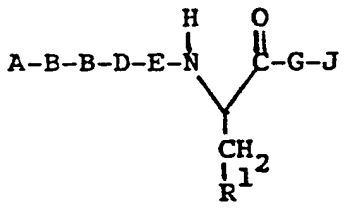


BOC-Phe-His-ACHPA-$C_6H_{12}O_5N$

BOC-Phe-His-ACHPA-$C_5H_{10}O_4N$

i-PRO$_2$C-His-His-ACHPA-NH-[(2S)-methyl]butyl
Et0$_2$C-Phe-His-ACHPA-NH-[(2S)-methyl]butyl
2(S)-hydroxy-3-phenylpropionyl-His-ACHPA-NH-[2S)-methyl]butyl
S-benzylthioacetyl-His-ACHPA-NH-[(2S)-methyl]butyl
Dibenzylacetyl-His-ACHPA-NH-[(2S)-methyl]butyl
Bis-(naphtylmethyl)acetyl-His-ACHPA-NH-[(2S)-methyl]butyl
Bis-(p-hydroxyphenylmethyl)acetyl-His-ACHPA-NH-[(2S)-methyl]butyl
[2-Phenylamino-3-phenylpropionyl-His-ACHPA-NH-[(2S)-methyl]butyl
2-Phenyloxy-3-phenylpropionyl]-His-ACHPA-NH-[(2S)-methyl]butyl
2-Phenylthio-3-phenylpropionyl-His-ACHPA-NH-[(2S)-methyl]butyl
1,3-Diphenylpropyloxcarbonyl-His-ACHPA-NH-](2S)-methyl-butyl
2-(1,3-diphenyl)propyloxcarbonyl-His-ACHPA-NH-[(2S)-methyl]butyl
2-Phenylthio-3-(1-naphthyl)propionyl-His-ACHPA-NH-[(2S)-methyl]butyl
[2-benzyl-2-(3,4-dihydroxy)benzyl]acetyl-His-ACHPA-NH-[(2S-methyl]butyl
[2-benzyl-2-(4-isopropyloxy)benzyl]acetyl-His-ACHPA-NH-[(2S)-methyl]butyl
BOC-Phe-His-[5-amino-6-cyclohexyl-4-hydroxy-2-isopropyl]hexanoyl 2(S)-aminobutane
BOC-Phe His-[5-amino-6-cyclohexyl-4-hydroxy-2-isobutyl]hexanoyl 2(S)-aminobutane
BOC-Phe-His-[5-amino-2-benzyl-6-cyclohexyl-4-hydroxy]hexanoyl 2(S)-aminobutane

Pro	Phe	His	Leu	Leu	Val	Tyr	
7	8	9	10	(11)	12	13	(14)



(I.)

As can be seen, a unique aspect and essential feature of the present invention is the substitution of the G component for the double amino acid sequence: Leu^{10}-Leu^{11} in the endogenous pig renin substrate. It is believed that substitution of this component for both leucine amino acids rather than just one leucine results in an improved substrate analogy due to the greater linear extent of the component as compared to a single leucine component. Thus, the component more closely approximates Leu-Leu in linear extent, and thereby provides a better "fit" to the renin enzyme.

The inhibitory peptides of the present invention may also be better appreciated in terms of substrate analogy from the following illustration of Formula I alongside the octapeptide sequence of a portion of the <u>human</u> renin substrate, which renin cleaves between Leu^{10} and Val^{11}:

Pro	Phe	His	Leu	Val	Ile	His	
7	8	9	10	(11)	12	13	(14)

cyanate, tosylate, and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups can be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl; and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

The present invention is also directed to combinations of the novel renin-inhibitory peptides of Formula I with one or more antihypertensive agents selected from the group consisting of diuretics, α and/or ß-adrenergic blocking agents, CNS-acting agents, adrenergic neuron blocking agents, vasodilators, angiotensin I converting enzyme inhibitors, calcium channel blockers, and other antihypertensive agents.

For example, the compounds of this invention can be given in combination with such compounds or salt or other derivative forms thereof as:

Diuretics: acetaz lamide; amil ride; bendroflumethiazide; benzthiazide; bumetanide; chlorothiazide; chlorthalidone; cyclothiazide; ethacrynic acid; furosemide; hydrochlorothiazide;

(α-[(tert.butylamino)methyl]-7-ethyl-2-benzofuran-methanol) (bufuralol);

(3-[3-acetyl-4-[3-(tert.butylamino)-2-hydroxypropyl]-phenyl]-1,1-diethylurea HCl) (celiprolol);

((±)-2-[2-[3-[(1,1-dimethylethyl)amino]-2-hydroxy-propoxy]phenoxy]-N-methylacetamide HCl) (cetamolol);

(2-benzimidazolyl-phenyl(2-isopropylaminopropanol));

((±)-3'-acetyl-4'-(2-hydroxy-3-isopropylaminopropoxy)-acetanilide HCl) (diacetolol);

(methyl-4-[2-hydroxy-3-[(1-methylethyl)aminopropoxy]]-benzenepropanoate HCl) (esmolol);

(erythro-DL-1-(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol);

(1-(tert.butylamino)-3-[O-(2-propynyloxy)phenoxy]-2-propanol (pargolol);

(1-(tert.butylamino)-3-[o-(6-hydrazino-3-pyridazinyl)-phenoxy]-2-propanol diHCl) (prizidilol);

((-)-2-hydroxy-5-[(R)-1-hydroxy-2-[(R)-(1-methyl-3-phenylpropyl)amino]ethyl]benzamide);

(4-hydroxy-9-[2-hydroxy-3-(isopropylamino)-propoxy]-7-methyl-5H-furo[3,2-g][1]-benzopyran-5-one) (iprocrolol);

((-)-5-(tert.butylamino)-2-hydroxypropoxy]-3,4-dihydro-1-(2H)-naphthalenone HCl) (levobunolol);

(4-(2-hydroxy-3-isopropylamino-propoxy)-1,2-benziso-thiazole HCl);

(4-[3-(tert.butylamino)-2-hydroxypropoxy]-N-methyliso-carbostyril HCl);

((±)-N-2-[4-(2-hydroxy-3-isopropyl aminopropoxy)-phenyl]ethyl-N'-isopropylurea) (pafenolol);

(3-[[(2-trifluoroacetamido)ethyl]amino]-1-phenoxy-propan-2-ol);

((1-tert.butylamino)-3-[(5,6,7,8-tetrahydro-cis-6,7-dihydroxy-1-naphthyl)oxy]-2-propanol) (nadolol);

((S)-1-(2-cyclopentylphenoxy)-3-[(1,1-dimethylethyl)-amino]-2-propanol sulfate (2:1)) (penbutolol);

(4'-[1-hydroxy-2-(amino)ethyl]methanesulfonanilide) (sotalol);

(2-methyl-3-[4-(2-hydroxy-3-tert.butylaminopropoxy)-phenyl]-7-methoxy-isoquinolin-1-(2H)-one);

(1-(4-(2-(4-fluorophenyloxy)ethoxy)phenoxy)-3-iso-propylamino-2-propanol HCl);

((-)-p-[3-[(3,4-dimethoxyphenethyl)amino]-2-hydroxy-propoxy]-ß-methylcinnamonitrile) (pacrinolol);

((±)-2-(3'-tert.butylamino-2'-hydroxypropylthio)-4-(5'-carbamoyl-2'-thienyl)thiazole HCl) (arotinolol);

((±)-1-[p-[2-(cyclopropylmethoxy)ethoxy]phenoxy]-3-(isopropylamino)-2-propanol) (cicloprolol);

((±)-1-[(3-chloro-2-methylindol-4-yl)oxy]-3-[(2-phenoxyethyl)amino]-2-propanol) (indopanolol);

((±)-6-[[2-[[3-(p-butoxyphenoxy)-2-hydroxypropyl]-amino]ethyl]amino]-1,3-dimethyluracil) (pirepolol);

(4-(cyclohexylamino)-1-(1-naphtholenyloxy)-2-butanol);

(1-phenyl-3-[2-[3-(2-cyanophenoxy)-2-hydroxypropyl]-aminoethyl]hydantoin HCl);

(3,4-dihydro-8-(2-hydroxy-3-isopropylaminopropoxy)-3-nitroxy-2H-1-benzopyran) (nipradolol);

<u>α and ß-Adrenergic Blocking Agents</u>:

((±)-1-tert-butylamino)-3-[o-[2-(3-methyl-5-iso-xazolyl)vinyl]phenoxy]-2-propanol) (isoxaprolol);

(1-isopropylamino-3-(4-(2-nitroxyethoxy)phenoxy)-2-propan 1 HCl);

((S)-1-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]octahydro-1H-indole-2-carboxylic acid HCl);

(N-cyclopentyl-N-(3-(2,2-dimethyl-1-oxopropyl)thiol-2-methyl-1-oxopropyl)glycine) (pivalopril);

((2R,4R)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid);

(1-(N-[1(S)-ethoxycarbonyl-3-phenylpropyl]-(S)-alanyl)-cis,syn-octahydroindol-2(S)-carboxylic acid HCl);

((-)-(S)-1-[(S)-3-mercapto-2-methyl-1-oxopropyl]-indoline-2-carboxylic acid);

([1(S),4S]-1-[3-(benzoylthio)-2-methyl-1-oxopropyl]-4-phenylthio-L-proline;

(3-([1-ethoxycarbonyl-3-phenyl-(1S)-propyl]amino)-2,3,4,5-tetrahydro-2-oxo-1-(3S)-benzazepine-1-acetic acid HCl);

(N-(2-benzyl-3-mercaptopropanoyl)-S-ethyl-L-cysteine) and the S-methyl analogue;

(N-(1(S)-ethoxycarbonyl-3-phenylpropyl)-L-alanyl-L-proline maleate) (enalapril);

N-[1-(S)-carboxy-3-phenylpropyl]-L-alanyl-1-proline;

N^2-[1-(S)-carboxy-3-phenylpropyl]-L-lysyl-L-proline (lysinopril);

Calcium Channel Blockers:

α-[3-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]-propyl]-3,4-dimethoxy-α-(1-methylethyl)benzene-acetonitrile (verapamil);

1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester (nifedipine);

2-(2,2-dicyclohexylethyl)piperidine (perhexiline);

N-(1-methyl-2-phenylethyl)- -phenylbenzenepropanamine (prenylamine);

Other Antihypertensive Agents: aminophylline; cryptenamine acetates and tannates; deserpidine; meremethoxylline procaine; pargyline; trimethaphan camsylate;
and the like, as well as admixtures and combinations thereof.

Typically, the individual daily dosages for these combinations can range from about one-fifth of the minimally recommended clinical dosages to the maximum recommended levels for the entities when they are given singly. Coadministration is most readily accomplished by combining the active ingredients into a suitable unit dosage form containing the proper dosages of each. Other methods of coadministration are, of course, possible.

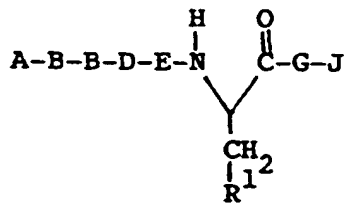
The novel peptides of the present invention possess an excellent degree of activity in treating renin-associated hypertension and hyperaldosteronism. Some of the peptides also have ACE inhibitor activity which may also make them useful for treating hypertension and congestive heart failure.

For these purposes the compounds of the present invention may be administered parenterally, by inhalation spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition to the treatment of warm-blooded animals such as mice, rats, horses, dogs, cats, etc., the compounds of the invention are effective in the treatment of humans.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

Thus, in accordance with the present invention there is further provided a pharmaceutical composition for treating renin-associated hypertension and hyperaldosteronism, or congestive heart failure comprising a pharmaceutical carrier and a therapeutically effective amount of a peptide of the formula:



(I.)

wherein A, B, D, E, R^1, G, and J have the same meaning as recited further above for Formula I; wherein all of the asymmetric carbon atoms have an S configuration, except for those in the B, D, and G substituents, which may have an S or R configuration; and a pharmaceutically acceptable salt thereof.

hypotensive dosage level and as a single dose, and there may result a transitory fall in blood pressure. This fall in blood pressure, if it occurs, indicates supranormal plasma renin levels.

An in vitro method which may be employed involves incubating a body fluid, preferably plasma, with a novel peptide of the present invention and, after deproteinization, measuring the amount of angiotensin II produced in nephrectomized, pentolinium-treated rats. Another in vitro method involves mixing the plasma or other body fluid with a novel peptide of the present invention and injecting the mixture into a test animal. The difference in pressor response with and without added peptide is a measure of the renin content of the plasma.

Pepstatin may be employed in the methods described above as an active control. See, e.g., U.S. Patent Nos. 3,784,686 and 3,873,681 for a description of the use of pepstatin in diagnostic methods of this type.

The novel peptides of the present invention may be prepared in accordance with well-known procedures for preparing peptides from their constituent amino acids, which will be described in more detail below.

A general method of preparation may be described in the following terms:

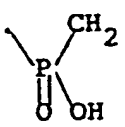
A method of preparing a peptide of Formula I, said peptide being comprised of from two to six amino acids identified as I through VI, amino acid (AA) I being the component G at the C-terminus of said peptide, and amino acid (AA) VI being at the

(F) deprotecting the tripeptide through hexapeptide formed in Step (E) to give the peptide of Formula I wherein A is hydrogen; and optionally

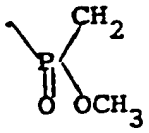
(G) treating the ttipeptide through hexapeptide formed in Step (H) with $R_a^2\text{-}X\text{-}\overset{\overset{O}{\|}}{\underset{\underset{R_b^2}{|}}{C}}\text{-}W$, where X, R_a^2, and R_b^2 are as defined above and W is an acid halide, anhydride, or other carboxyl activating group, to give the peptide of Formula I wherein A is other than hydrogen;

said method also comprising, where necessary, protection of sidechain substituents of the component amino acids AA I through AA VI, with deprotection being carried out as a final step; said method also comprising any combination of the steps set out above, whereby the amino acids I through VI and substituents A, G, and J, are assembled in any desired order to prepare the peptide of Formula I; and said method also comprising employment of the steps set out above in a solid phase sequential synthesis, whereby in the initial step the carboxyl group of the selected amino acid is bound to a synthetic resin substrate while the amino group of said amino acid is protected, followed by removal of the protecting group, the succeeding steps being as set out above, the peptide as it is assembled being attached to said synthetic resin substrate; followed by a step of removing the peptide of Formula I from said synthetic resin substrate; and after removal of the peptide of Formula I from said synthetic resin substrate, the step of teating said ester thereof in accordance with the pr cedures d scribed in Step (C)

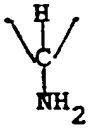
involve addition of the isostere component in a protected form. For example, the following reactive groups would require such protection:



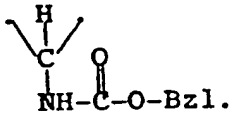
as



; and

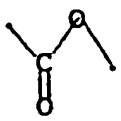


as

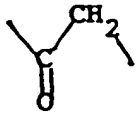


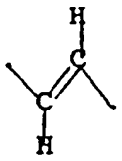
Such protecting groups may be removed as a final or near final step, for example, by base hydrolysis in the former case, or by hydrogenation in the latter.

Preparation of the particular isostere components may be carried out in accordance with procedures described below and in the literature cited particularly as follows:

A.  (depsipeptides)

Ondetti et al., Chemistry and Biology of Peptides, ed. J. Meienhofer, Ann. Arbor Science pp. 525-531, 1972.

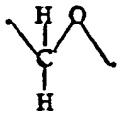
B.  (ketomethylene)

E.  (ethylene)

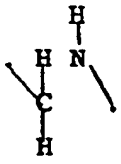
(1) Natarajan et al., Id.

(2) Hann et al., J. Chem. Soc. Chem. Comm., 234-235, 1980.

(3) Cox et al., J. Chem. Soc. Chem. Comm., 799-800, 1980.

F.  (methylene ether)

Ondetti et al., Id.

G.  (methylene aza, or reduced isostere)

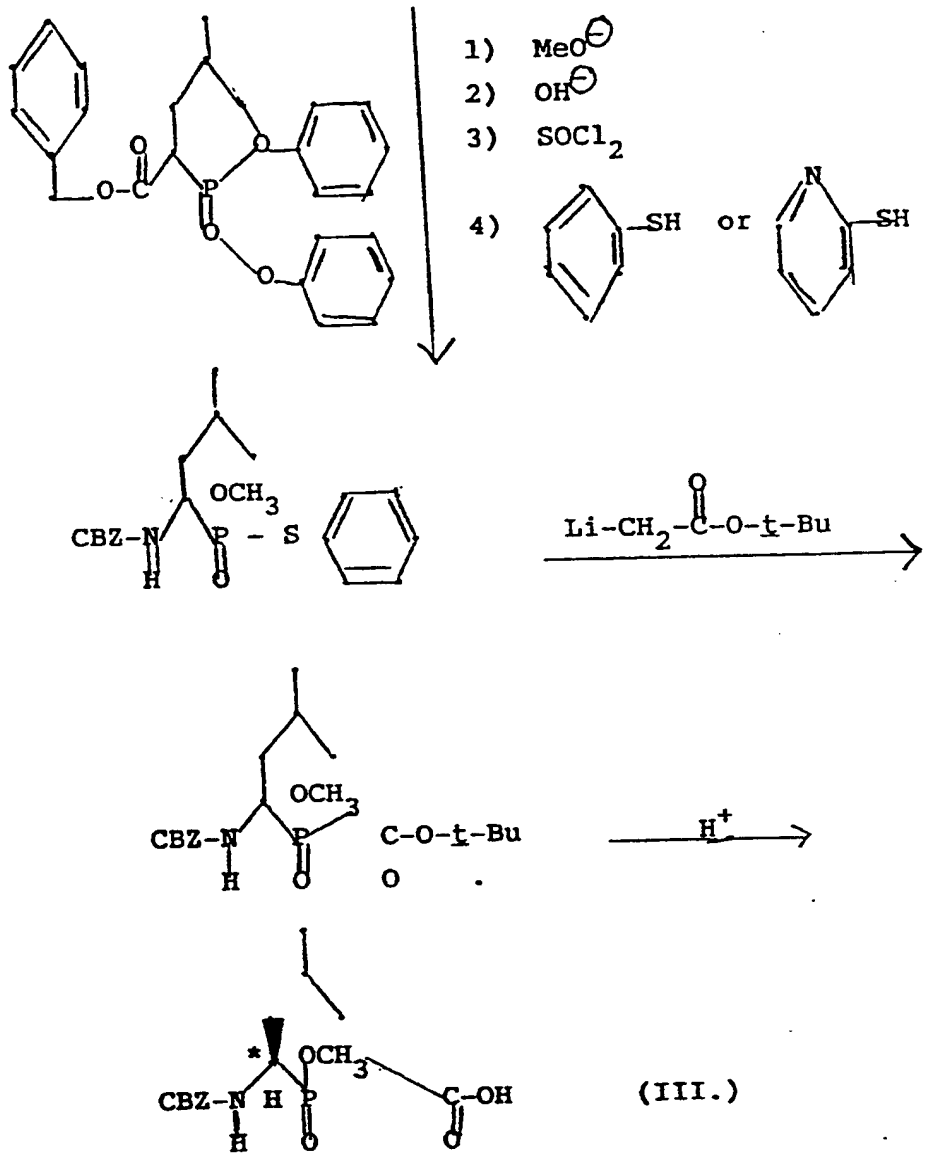
(1) Van Lommen et al., Id.

(2) Atherton et al., J. Chem. Soc. (C), 3393-3396, 1971.

(3) Parry et al., Chemistry and Biology of Peptides, ed. J. Meienhofer, Ann Arbor Science, pp. 541-544, 1972.

(4) Hudson et al., Int. J. Peptide Protein Res. 15: 122-129, 1979.

(5) Frank and Desiderio, Anal. Biochem. 90: 413-419, 1978.

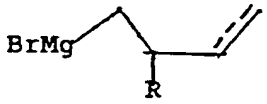


(mixture of two pairs of diastereomers; two isomers at *; active isomer indicated).

which can be incorporated into the synthesis for the overall peptide of the present invention, or converted to the α-BOC derivative by hydrogenation over Pd/C catalyst, followed by treatment with

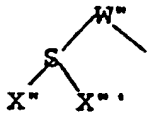
(active isomer shown; other isomers obtained as well)

Incorporation of (IV.) or its N-BOC analog proceeds as for (III.) above, with removal of the methyl phosphinate ester by hydrolysis (alkaline) to give the free phosphinate. The active isomer shown at * has the side chains in the relative configuration of the dipeptide that they mimic. For synthesis of



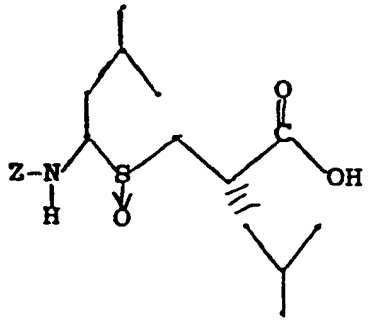
, see Jennings-White and Almquist, Id.

J.

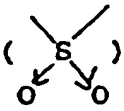


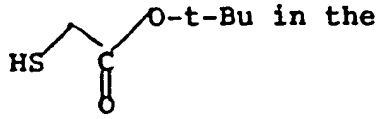
Morton et al., Id.

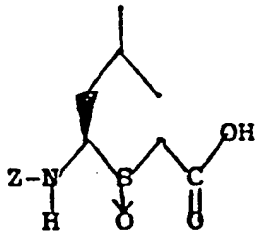
For example, the compound:

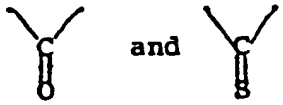


can be prepared in accordance with the following scheme:

The sulfone () can be obtained using excess m-Cl-perbenzoic acid.

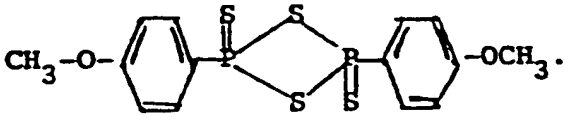
Use of  second step gives as the final product:



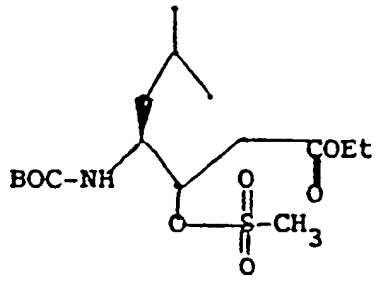
K. 

Prepared from the alcohol; see Rich et al., Biochem. Biophys. Res. Comm. 104:1127-1133, 1982.

Conversion of the ketone to the thioketone is with use of:



L. 

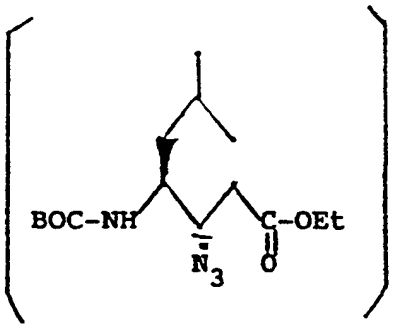


greater than 95% yield

greater than 95% purity (TLC, NMR)

$CDCl_3$ | $(nBU)_4N^+N_3^-$

1 eq., 45°C, 18 hr.



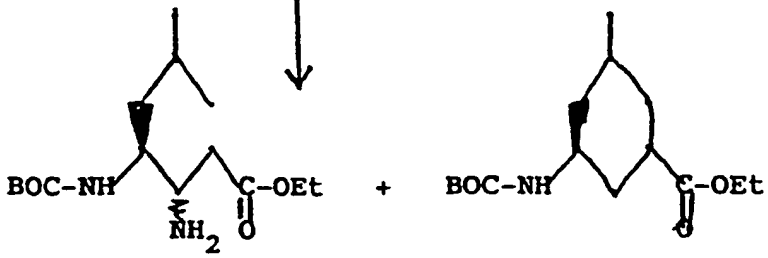
+ 20% elimination + $(nBu)_4N^+X$

Aqueous workup

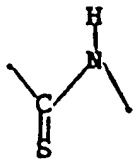
100% elimination

$CHCl_3/EtOH$ | PtO_2/H_2

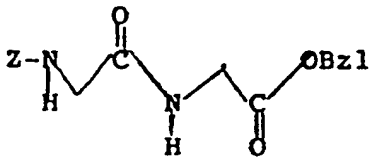
40 lbs., 4 hr.



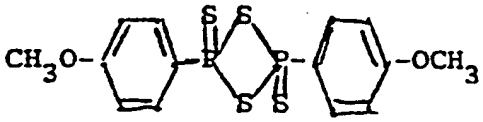
M.

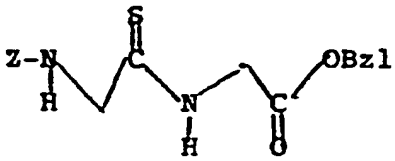


Obtained from the amide according to the method described by Clausen et al., Seventh American Peptide Symposium, 1981:

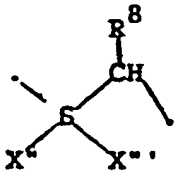


80°C; 0.5 Hour,





N.



(1) Natarajan et al., Id.

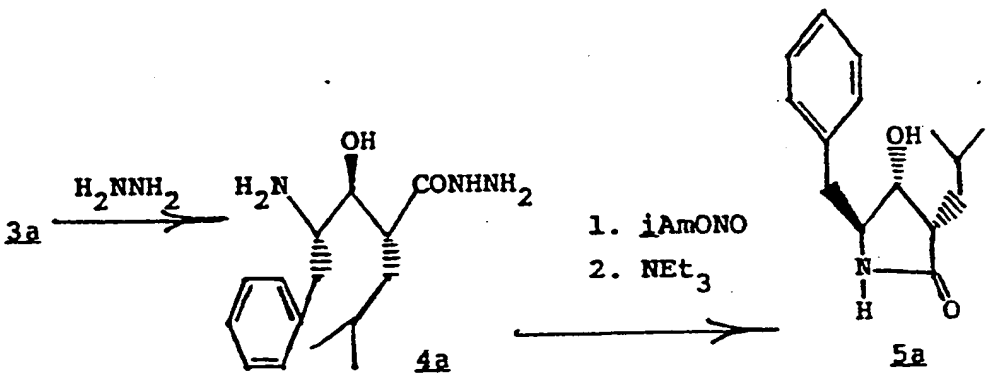
(2) Fok and Yankellov, Id.

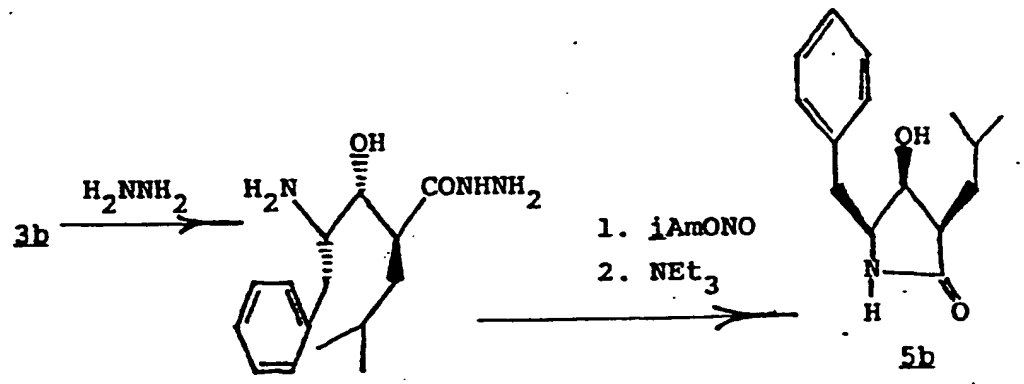
(3) Spatola et al., Id.

(4) Spatola and Bettag, Id.

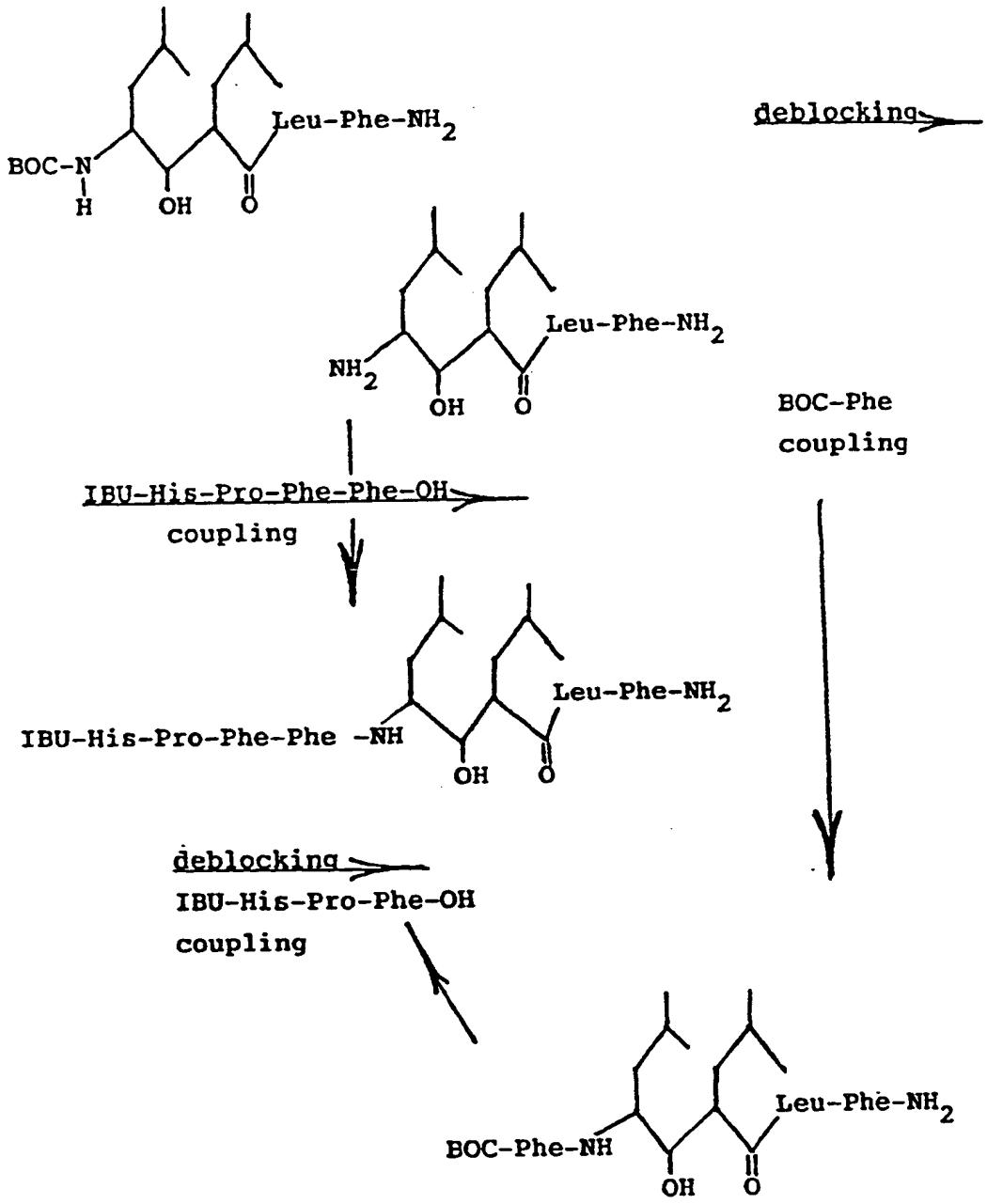
(5) Spatola et al, Proceedings of the Seventh American Peptide Symposium, ed. E. Gross and D. H. Rich, pp. 613-616, 1981.

The configurations of the chiral centers can be established as follows: treatment of the phthalimido methyl esters 3a and 3b with excess hydrazine gives the respective amino acyl hydrazides 4a and 4b, which are then converted in a two step/one pot procedure to the corresponding lactams 5a and 5b, to which stereochemical assignments can be made based on PMR analysis. These reactions may be illustrated as follows:





Alternatively, the benzyl ester 6, rather than the methyl ester, may be used to form the ketone silylacetal 7, which can then be reacted with phthalyl phenylalanine aldehyde and phthalyl leucine



III. Where the Component G Is the Amino Acid Statine Itself: it may be prepared in accordance with the procedure described by Rich et al., J. Org. Chem. 43: 3624, 1978.

TABLE I

Abbreviated Designation	Amino Acid
Ala	L-alanine
Arg	L-arginine
Gly	L-glycine
His	D or L-histidine
Ile	L-isoleucine
Leu	L-leucine
Lys	L-lysine
Met	L-methionine
Orn	L-ornithine
Phe	L-phenylalanine
Ser	L-serine
Sar (N-methylglycine)	L-sarcosine
Thr	L-threonine
Trp	L-tryptophan
Tyr	L-tyrosine
Val	L-valine

Abbreviated Designation	Protecting Groups
BOC	tert-butyloxycarbonyl
CBZ	benzyloxycarbonyl
DNP	dinitrophenyl
OMe	methyl ester

Abbreviated Designation	Activating Groups
HBT	1-hydroxybenzotriazole

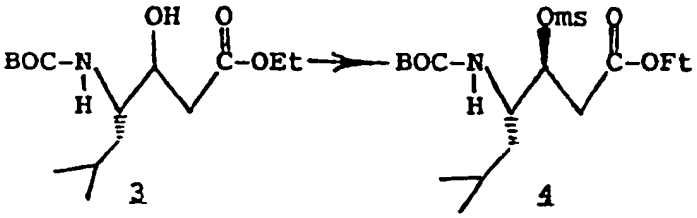
ether and stannic chloride. The Friedel-Crafts reaction is continued until the resin contains 0.5 to 5 mmoles of chlorine per gram of resin.

The amino acid selected to be the C-terminal amino acid of the linear peptide is converted to its amino protected derivative. The carboxyl group of the selected C-terminal amino acid is bound covalently to the insoluble polymeric resin support, as for example, as the carboxylic ester of the resin-bonded benzyl chloride present in chloromethyl-substituted polystyrene-divinylbenzene resin. After the amino protecting group is removed, the amino protected derivative of the next amino acid in the sequence is added along with a coupling agent, such as dicyclohexylcarbodiimide. The amino acid reactant may be employed in the form of a carboxyl-activated amino acid such as ONP ester, an amino acid azide, and the like. Deprotection and addition of successive amino acids is performed until the desired linear peptide is formed.

The selection of protecting groups is, in part, dictated by particular coupling conditions, in part by the amino acid and peptide components involved in the reaction.

Amino-protecting groups ordinarily employed include those which are well known in the art, for example, urethane protecting substituents such as benzyloxy-carbonyl (carbobenzoxy), p-methoxycarbobenzoxy, p-nitrocarbobenzoxy, t-butyoxycarbonyl, and the like. It is preferred to utilize t-butyloxycarbonyl (BOC) for protecting the α-amino group in the amino acids undergoing reaction at the carboxyl end of said amino acid. The BOC protecting group is

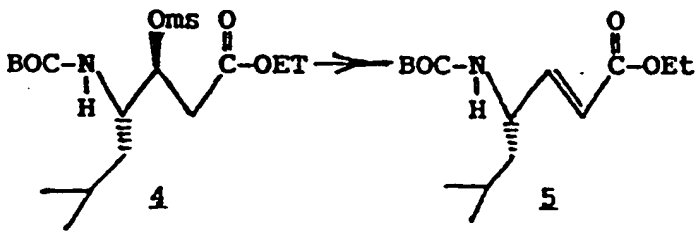
Step A.



3 4

To an ice cold, stirred solution of BOC-statine ethyl ester 3 (2.60 g, 8.57 mmole) in 10 ml of pyridine is added via syringe 0.66 ml (8.57 mmole) of trifluoromethane sulfonyl chloride. Within minutes, pyridinium hydrochloride precipitates from solution. The reaction mixture is protected from moisture and allowed to stand at room temperature overnight. The reaction mixture is filtered and the filtrate concentrated to give a light orange oil. The crude product is filtered through 5-10 g of silica gel (ether elution) to give 3.0 g of a pale yellow oil which is used without further purification. Compound 4 is prone to hydrolysis to 3 and must be used without delay.

Step B.

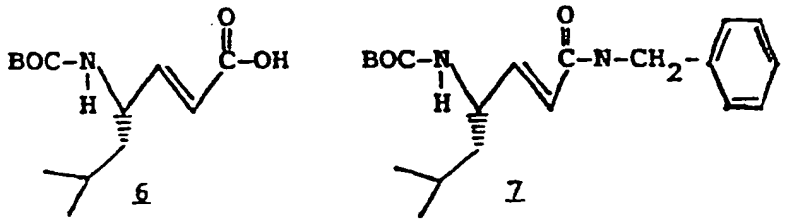


4 5

1,5-Diazabicyclo[4.3.0]non-5-ene (DBN) (0.94 ml, 7.54 mmole) is added in one portion to a

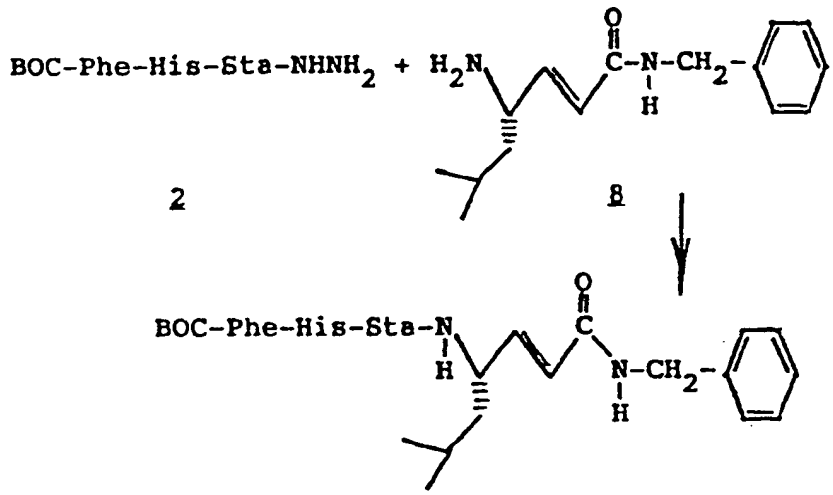
The aqueous phase is acidified with 10% citric acid and extracted with ether and chloroform. The combined organic extracts are washed with brine and dried (Na_2SO_4). Concentration under reduced pressure affords 1.46 g of 6.

Step D.



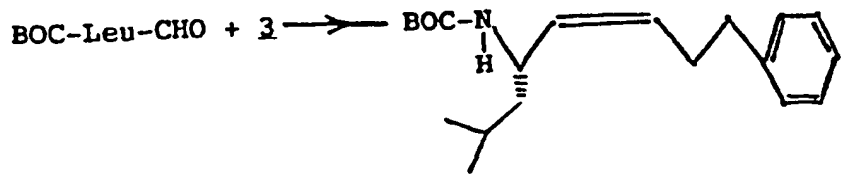
The acid 6 (400 mg, 1.6 mmole) is dissolved in 4 ml of methylene chloride under nitrogen. N-methylmorpholine (0.18 ml, 1.6 mmole) is added and the solution is cooled to -5°C. Isobutylchloroformate (0.21 ml, 1.6 mmole) is added and after 15 minutes, 0.22 ml (1.92 mmole) of benzylamine is added to the reaction mixture. After 30 minutes at -5°C the reaction mixture is warmed to room temperature, stirred 1 hour more and diluted with 70 ml of methylene chloride. The organic phase is washed in succession with 10% citric acid (2 x 30 ml), saturated sodium bicarbonate solution (2 x 30 ml), and brine. The organic extracts are dried (Na_2SO_4) and concentrated to yield 460 mg of a white solid identified as 7.

Step G.



The hydrazide 2 (240 mg, 0.42 mmole) is dissolved in 2 ml of dry dimethylformamide under nitrogen. The solution is cooled to -20°C and the pH of the reaction mixture adjusted to approximately 0.5-1.0 with tetrahydrofuran saturated with hydrogen chloride. Isoamyl nitrite is then added in 50 µl increments at 15-20 minute intervals until a positive potassium iodide-starch test is obtained (250 µl total). The amine salt 8 (190 mg, 0.67 mmole) is dissolved in 2 ml of dimethylformamide and added to the reaction mixture. After addition is complete, the pH of the reaction mixture is adjusted to 7.5-8.0 with triethylamine and the reaction mixture is allowed to stir at -20°C for 20 hours. The reaction is filtered and the filtrate concentrated. The resulting residue is partitioned between ethyl acetate and water. The organic phase is washed in succession with 10% citric acid solution (2 x 50 ml), 50% sodium bicarb nate solution (2 x 50 ml), and

Step B.

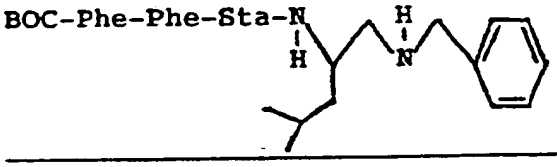


1 4

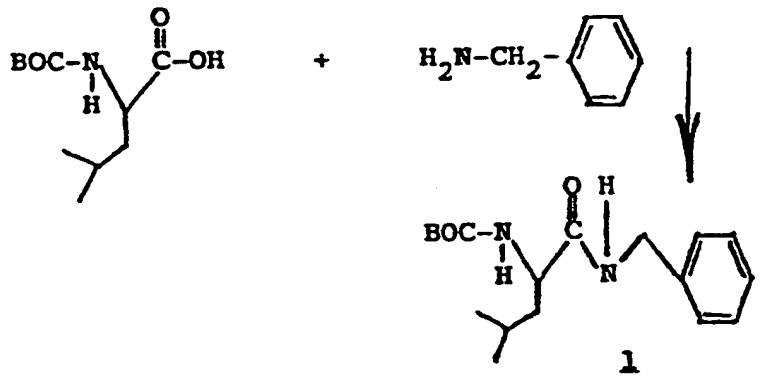
A rapidly stirred suspension of 3 (1.82 g, 3.95 mmole) in 15 ml of dry tetrahydrofuran is treated dropwise under nitrogen at 0°C with n-butyl lithium (1.4N, 2.82 ml, 3.95 mmole). The reaction mixture, which becomes homogeneous and colors to dark brown, is cooled to -78°C and treated with 5 ml of tetrahydrofuran containing 0.5 g (2.32 mmole) of BOC-leucine aldehyde 1. After four hours at -78°C the reaction mixture is warmed to -10°C for 1 hour and then quenched with saturated ammonium chloride solution. The reaction is partitioned between ether and brine. The organic phase is then washed with 10% citric acid solution (3 x 20 ml), 50% sodium bicarbonate solution (3 x 20 ml), and brine. Rotoevaporation of the dried (Na_2SO_4) extracts affords 0.42 g of crude product as a yellow oil. The analytical sample is obtained by chromatography of the crude product on silica gel (hexane-ethyl acetate 9:1 elution).

identical reaction conditions as described in Example 1, Step G]. The amine salt 5 (160 mg, 0.63 mmole) is then added [and the reaction is carried out and worked-up as described in Example 1, Step G]. The analytical sample (70 mg) is obtained after silica gel chromatography (80:10:1 $CHCl_3$-ethanol-ammonia elution) as a pale yellow solid.

EXAMPLE 3



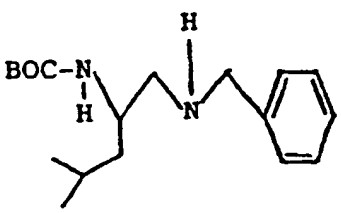
Step A.



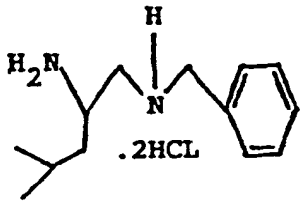
1

N-Methylmorpholine (9.14 ml, 83.2 mmole), and BOC-leucine hydrate (20.0 g, 83.2 mmole) are dissolved in CH_2Cl_2 (200 ml) and the solution cooled to -5°C. Isobutylchloroformate (10.8 ml, 83.2 mmole) is added and the solution stirred 15 min. Benzylamine (10.9 ml, 99.8 mmol) is added and the solution stirred 15 min. The solution is warmed to

Step C.



2

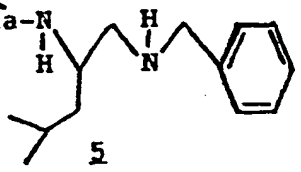


3

Protected amine 2 (540 mg, 1.8 mmol) is dissolved in ethyl acetate (10 ml). The solution is cooled to 0°C, saturatad with HCl (g), and stirred 15 min. The solvent is removed in vacuo. The residue is treated with ethyl acetate and restripped (4X) to give a quantitative (490 mg) yield of 3 as an off-white solid.

Step D.

3 + BOC-Phe-Phe-Sta-OH → BOC-Phe-Phe-Sta-

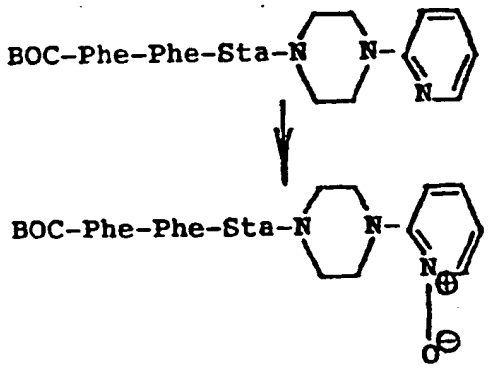


5

BOC-Phe-Phe-Sta-OH (240 mg, 0.421 mmole), diamine dihydrochloride 3 (130 mg, 0.466 mmole), 1-hydroxybenzotriazole hydrate (HBT) (62.9 mg, 0.466 mmole), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (895 mg, 0.466 mmole) are dissolved in degassed dimethylformamide (4 ml) under a nitrogen atmosphere. Th pH of the

mg, 0.2 mmole), 2-pyridylpiperazine 33.5 µl, 35 mg, 0.22 mmole) diphenylphosphonylazide (47.5 µl, 60.7 mg, 0.22 mmole), and sodium bicarbonate (84 mg, 1 mmole). The resulting suspension is protected from moisture and stirred at 0° for 12 hours. More diphenylphosphonylazide is added (47.5 µl, 0.22 ml) and stirring continued at 0°C. After 2 days the reaction mixture is filtered and the filtrate concentrated in vacuo. The residue is chromatographed on silica gel (90:10:1:0.1 chloroform/methanol/water/acetic acid elution) to give 125 mg of the analytical sample as a white solid.

Step B.



The tri-peptide of Step A. (41 mg, 0.06 mmole) is dissolved in 5 ml of chloroform and the resulting solution is treated with 20 mg of tech. grade m-chloroperbenzoic acid (85%). The reaction mixture is allowed to stand for 19 hours at room temperature and then the solvent is removed under r duced pressure. The residue is chr matographed on silica gel (chl roform-ethanol-ammonia 80:10:1 elution). The material with R_f value of 0.24 is

$R^{1'}$ is alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkyl alkyl, aryl alkyl, heterocyclic, heterocyclic each of which may be substituted with up to three members selected from alkyl, halo, amino and alkoxy groups.

n' is 0 or 1.

$R^{2'}$ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkyl alkyl, aryl, aryl alkyl, heterocyclic, heterocyclic alkyl, each of which may be substituted with up to three members selected from alkyl, hydroxy, halo, amino, alkylamino, dialkylamino, and alkoxy.

$R^{3'}$ is OH, NH_2, $NHR_a^{3'}$, $NR_a^{3'}N_b^{3'}$, $OR_c^{3'}$

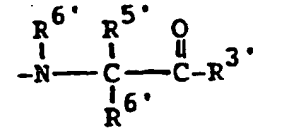
where $R_a^{3'}$, $R_b^{3'}$, and $R_c^{3'}$ are separately alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkyl alkyl, aryl, aryl alkyl, heterocyclic, heterocyclic alkyl, each of which may be substituted with up to three members selected from amino, alkyl amino, dialkyl amino, trialkyl ammonium, hydroxy, alkoxy, aryloxy, aryl alkoxyl, or halo.

$R_c^{3'}$ may also be $R_d^{3'}$-CO-V'-$CR_e^{3'}R_f^{3'}$ wherein $R_d^{3'}$ is alkyl or aryl; $R_e^{3'}$ and $R_f^{3'}$ are hydrogen or alkyl; V' is -O- or -NH-.

$R^{4'}$ is hydrogen, alkyl, alkenyl, alkynyl, aryl, arylalkyl, each f which may be substituted with up to three memb rs selected from amino, alkyl amino, dialkyl amin , trialkyl

$R^{8'}$ is hydrogen, methyl and cycloalkyl including cyclopentyl and indanyl, such that when $R^{8'}$ is cycloalkyl, $R^{6'}$ and $R^{7'}$ are both hydrogen.

$R^{9'}$ is hydroxy, $OR_a^{3'}$, $-NH_2$, $-NHR_a^{3'}$, $NR_a^{3'}R_b^{3'}$, where $R_a^{3'}$ and $R_b^{3'}$ are as defined above, such that when A' and B' are both absent, $R^{9'}$ can be



and pharmaceutically acceptable salts thereof.

In the above definitions, the terms alkyl or alk, alkenyl, alkynyl, include hydrocarbon groups of up to 8 carbon atoms which groups may be straight or branched chain. Preferred alkyl or alk groups have 1-4 carbon atoms. Preferred alkenyl and alkynyl groups have 3 to 6 carbon atoms.

The term halo means fluoro, chloro, bromo and iodo.

The term aryl represents hydrocarbon aryl of up to 10 carbon atoms exemplified by phenyl, naphthyl, biphenyl and cycloalkyl-fused derivatives thereof such as indanyl and tetralinyl.

The term heterocyclic represents substituted and unsubstituted 5- or 6-membered ring containing from one to three heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur having varying degrees of unsaturated wherein the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen atom be quaternized, and including any bicyclic group in which any f the abov heterocyclic rings is fused to a benzene ring. Heter cyclic

c) compounds of Formula I, I°, I^a and I^b where 1.) X' is OR_4' and R_4' is other than H, and preferably is C_1-C_6 alkyl or 2.) R_4' in the E° unit is other than H, and preferably is C_1-C_6 alkyl.

The peptides of the present invention may also be described in terms of common amino acid components and closely related analogs thereof, in accordance with formula (I):

$$A°-B°-D°-E°-G°$$
$$(I)$$

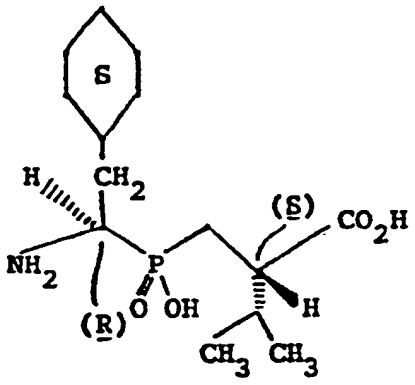
werein A° has the same meaning as described above: A° and B° can each be, for example, Ala, Leu, Phe, Tyr, Trp, Met, HomoPhe, BishomoPhe, HomoTyr, HomoTrp, 3-(1-Naphthyl)Ala, 3-(2-Naphthyl)Ala, 5-MethoxyTrp, N-MethylPhe, N-Methyl-HomoPhe, α-methylPhe.

It will be understood that closely related analogs of the above common amino acids, for examples, aliphatic amino acids in addition to Ala, Val, Leu, and Ile, such as alpha amino butyric acid (Abu) are included in the broad description of the peptides of the present invention represented by Formula (I) and its definitions.

In the above definitions, when A° is R°CO, and A° is alkyl, aryl, aryl alkyl, heterocyclic or heterocyclic alkyl, these groups being optionally substituted by amino, C_2-C_6 alkanoylamino, hydroxy, alkyl, alkoxy, alkoxycarbonyl, halo, or nitro; and B° is absent and D° is

Preferred G° substituents include -OH, -OEt, $-NH_2$.

The amino acid units have asymmetric centers and occur as racemates, racemic mixtures and as individual diastereomers. All isomeric forms are included in the present peptides. In general, the preferred chiral forms of amino acid units B° and D° are the (L) forms. The stereocenters present in the E° unit of the peptides of Formula I° are in general of the chirality which corresponds to the naturally-occurring (L) amino acids. Thus, for example, the unit E° of the formula below possesses the stereochemistry shown in the preferred form:



The following are illustrative examples of Formula I° peptides:

1. [N-(N-(N-carbobenzoxy-2-amino-3-(1-naphthyl)-propionyl)histidyl)-1-amino-2-cyclohexylethyl] 2-carboxy-4-methylpentylphosphinic acid
2. [N-(N-(N-carbobenzoxy-2-amino-3-(1-naphthyl)-propi nyl)histidyl)-1-amino-2-cyclohexylethyl] 2-carboxy-3-methylbutylphosphinic acid

14. [N-(N-carbobenzoxy-histidyl)-1-amino-2-cyclohexyl-ethyl] 2-carboxy-4-methylpentylphosphinic acid
15. [N-(phenylalanyl)-1-amino-2-cyclohexylethyl] 2-carboxy-3-methylbutylphosphinic acid
16. [N-(lysyl)-1-amino-2-cyclohexylethyl] 2-carboxy-3-methylbutylphosphinic acid
17. [N-(Nε-1-naphthyloxyacetyl-lysyl)-1-amino-2-cyclohexylethyl] 2-carboxy-3-methylbutylphosphinic acid
18. Methyl [N-(N-(N-carbobenzoxy-2-amino-3-(1-naphthyl)propionyl)histidyl)-1-amino-2-cyclohexyl-ethyl] 2-carboxy-4-methylpentylphosphinate
19. Methyl [N-(N-(N-carbobenzoxy-2-amino-3-(1-naphthyl)propionyl)histidyl)-1-amino-2-cyclohexyl-ethyl]carbomethoxymethylphosphinate
20. Ethyl [N-(N-3-phenylpropionyl-phenylalanyl)-1-amino-2-cyclohexylethyl] 2-carbomethoxy-3-methyl-butylphosphinate

The Formula I° compounds include many which bear acidic and/or basic groups; pharmaceutically acceptable salts of these compounds are included. Among the useful acid addition salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentane-propionate, digluconate, dodecylsulfate, ethane-sulfonate, fumarate, glucoheptanoate, glycero-phosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxy-ethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinat , persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thio-

The novel peptides of Formula I° possess an excellent degree of activity in treating hypertension and congestive heart failure. The Formula I° compounds also are expected to be orally active.

For these purposes the compounds of Formula I° may be administered orally, parenterally, by inhalation spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition to the treatment of warm-blooded animals such as mice, rats, horses, dogs, cats, etc., the compounds of the invention are effective in the treatment of humans.

The pharmaceutical compositions containing Formula I° peptides may be provided in oral dosage forms e.g. tablets, capsules, solutions, dispersions, etc., the oral formulations are prepared using conventional procedures and compounding ingredients e.g. carriers, diluents, etc. The compositions may also be in the form of a sterile injectable preparation, for example as a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acc ptabl vehicles and s lvents that may be employed are water, Ringer's solution and isotonic

excretion, drug combination and the severity of the particular disease undergoing therapy.

Thus, in accordance with the present invention there is further provided a pharmaceutical composition for treating hypertension and congestive heart failure, comprising a pharmaceutical carrier and a therapeutically effective amount of a peptide of the Formula I°.

Also, in accordance with the present invention there is still further provided a method of treating hypertension and congestive heart failure, comprising administering to a patient in need of such treatment, a therapeutically effective amount of a peptide of the Formula I°.

The renin inhibitory peptides of Formula I° may also be utilized in diagnostic methods for the purpose of establishing the significance of renin as a causative or contributory factor in hypertension or congestive heart failure in a particular patient. For this purpose these peptides may be administered in a single dose of from 0.1 to 10 mg per kg of body weight.

Both in vivo and in vitro methods may be employed. In the in vivo method, a novel peptide of the present invention is administered to a patient, preferably by intravenous injection, although other routes of parenteral administration are also suitable, at a hypotensive dosage level and as a single dose, and there may result a transitory fall in blood pressure. This fall in blood pressure, if it occurs, indicates supranormal plasma renin levels.

Some of the peptides of Formula I, particularly those of Formula I° also have

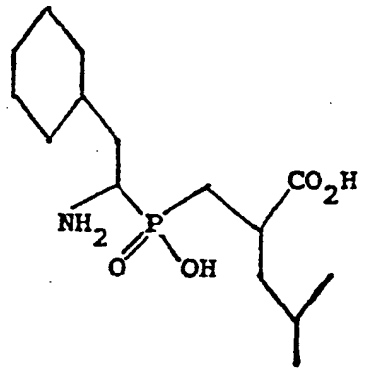
of dicyclohexylcarbodiimide/1-hydroxybenzotriazole and of disuccinimido oxallate (K. Takeda et al., Tetrahedron Lett., 24, 4451-54 (1983)). In many cases, both carboxylic and phosphonic (or phosphinic esters) may be hydrolyzed along with amino protecting groups as the last step in the synthesis. In these cases, treatment of the phosphorus-containing peptide analog with 30% hydrobromic acid in acetic acid, with 6N hydrochloric acid, or with aqueous sodium hydroxide, followed by purification of the resulting deprotected material by ion-exchange chromatography or reverse-phase HPLC, provides the desired product. In instances where the phosphorus-containing component E possesses a carboxyl-terminal amide function, the fully coupled material A°-B°-D°-E° may be treated with 1 equivalent of lithium hydroxide (0.1 N) to hydrolyze selectively an ester function in E°. Standard coupling procedures may then be used to couple the resulting free carboxylic acid to an appropriate amine. This is followed by removal of the remaining protecting groups as described above. Alternatively the amide formation may be carried out prior to coupling of the component E° to the A°-B°-D° unit.

Preparation of the phosphorus-containing components E are carried out as illustrated in the examples to follow.

The 1-aminoalkylphosphonous acids used as starting materials in the examples below are prepared as illustrated in the examples and can be resolved to give optically active materials by the method of Baylis et al. (J. Chem. Soc. Perkin Trans 1, 2845-53 (1984)). C mpounds deriv d from both the optically

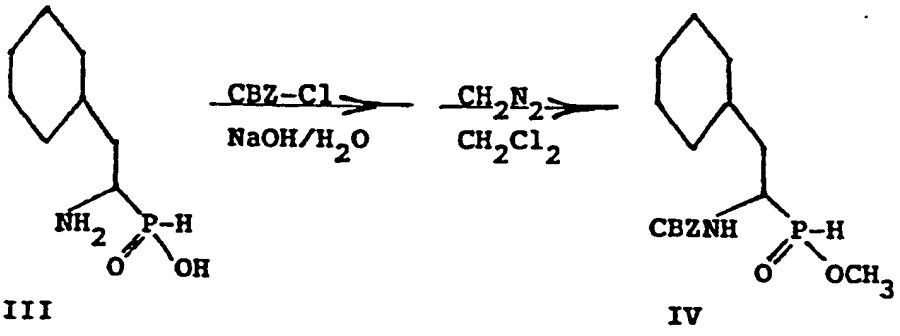
It will be understood that the following schemes outline representative examples of the preparation of peptides of Formula I and that similar peptides possessing alternative substituents can equally well be prepared by the routes outlined.

The component E° may be, for example, a phosphinic acid of the Formula II.



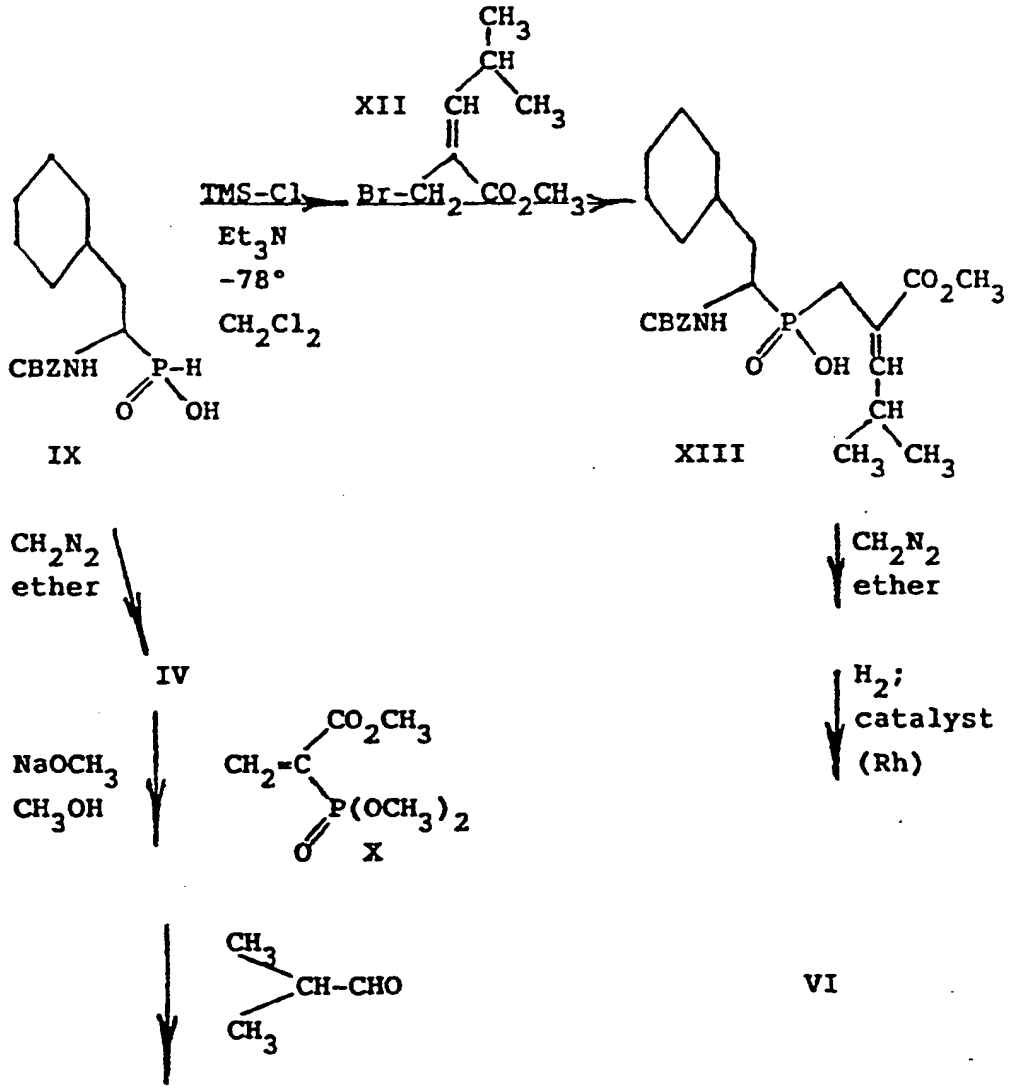
II

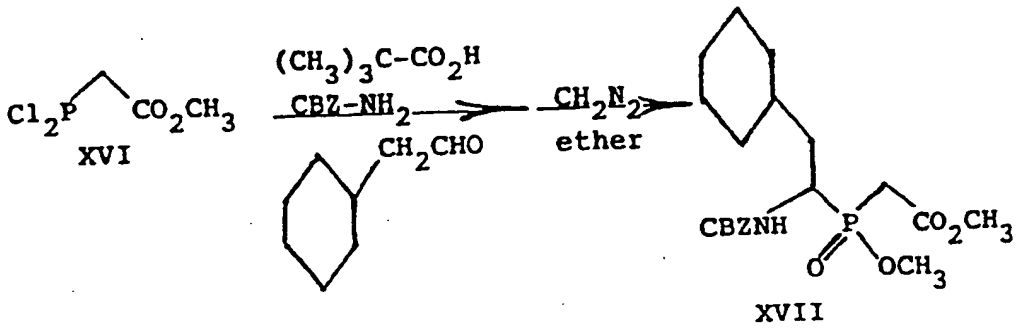
Peptide VIII containing II may be prepared as outlined in the scheme below:



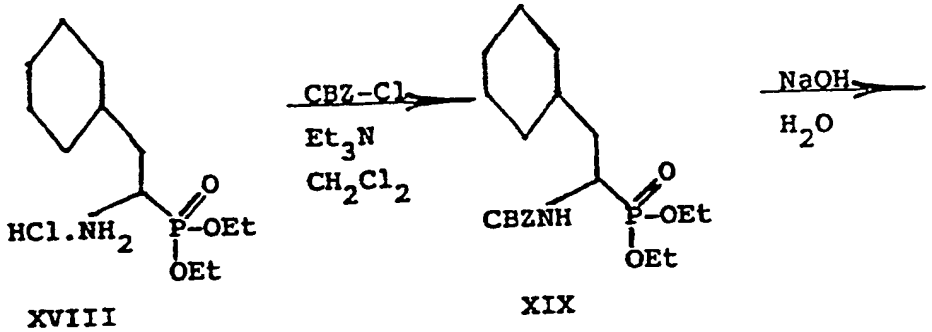
III IV

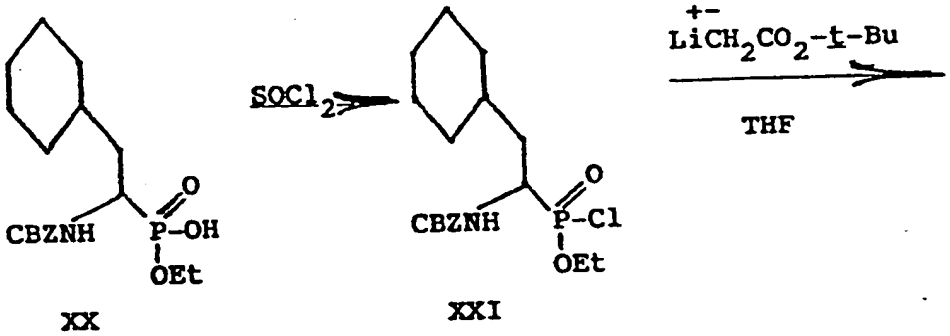
An alternative procedure for preparation of phosphinate VI is illustrated in the Scheme below:



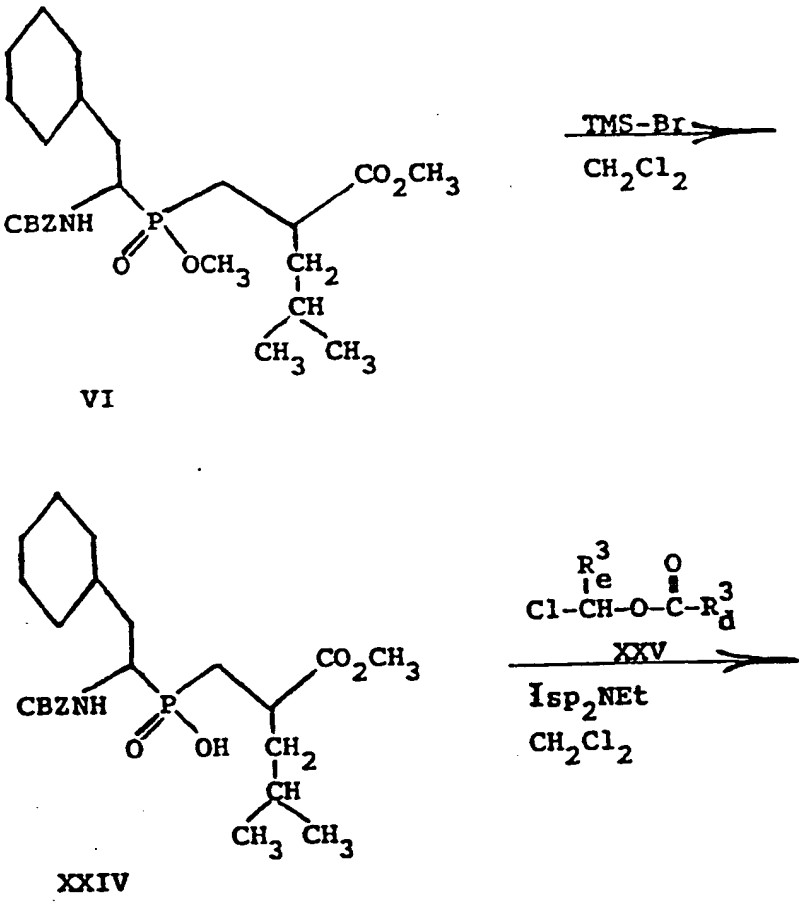

Cl2P
CO2CH3
XVI
(CH3)3C-CO2H
CBZ-NH2
CH2CHO
CH2N2
ether
CBZNH
P
CO2CH3
O
OCH3
XVII

Route B:


HCl.NH2
P-OEt
OEt
O
XVIII
CBZ-Cl
Et3N
CH2Cl2
CBZNH
P-OEt
OEt
O
XIX
NaOH
H2O


CBZNH
P-OH
OEt
O
XX
SOCl2
CBZNH
P-Cl
OEt
O
XXI
+-
LiCH2CO2-t-Bu
THF

Peptide XXVII containing such a component E may be prepared as outlined in the following scheme:



Melting points were recorded on a Thomas-Hoover melting point apparatus and are uncorrected as are all boiling points. ^1H NMR spectra were taken on a Varian XL-300 FT spectrometer. Chemical shifts are reported in ppm downfield from tetramethylsilane as internal standard. IR spectra were recorded on a Perkin-Elmer Model 297 spectrometer. Optical rotations were measured with a Perkin Elmer 141 automatic polarimeter in the solvents indicated. Mass spectra (MS) were taken on a Varian 731 spectrometer at 70 eV. Those marked FAB were taken by using the fast atom bombardment method.

The following examples illustrate preparation of representative compounds, particularly those of Formula I°. All temperatures are in °C unless otherwise noted.

EXAMPLE 1A

Cyclohexylacetaldehyde

A suspension of 100 g (0.46 moles) of pyridinium chlorochromate and 100 g of celite in 800 ml of methylene chloride was stirred vigorously while 38 g (0.3 moles) of 2-cyclohexylethanol in 200 ml of methylene chloride was added all at once. The reaction turned dark immediately and became mildly exothermic. After 1 hour, 1000 ml of ether was added and the reaction mixture was filtered through a bed of silica gel (ca. 250 g) on a fritted glass disk. The pad was rinsed with an additional liter of ether. The combined filtrates were reduced in volume to approximately 200 ml and th solution was washed with 2 x 40 ml of 6N HCl, 1 x 50 ml f saturated

The flask was then immersed in an oil bath preheated to 115°C. After 1 hour the reaction was almost homogeneous. After a total of 3 hours at 115°C, the reaction mixture was cooled to 0°C in an ice bath. The solution was washed with 1 x 200 ml and 2 x 100 ml of hexanes. The hexanes wash was discarded and the remaining aqueous acid solution was evaporated to dryness on a rotary evaporator. The resulting semi-crystalline foam was dissolved in 125 ml of absolute ethanol and cooled to 0°C in an ice bath. Propylene oxide (50 ml) was slowly added and a white precipitate was formed. The reaction mixture was allowed to warm to room temperature of its own accord while stirring. After a total of 18 hours, the slurry was cooled to 0°C again, and the solid filtered off. The solid was washed with 100 ml more of ice-cold ethanol and dried to afford 11.98 g (53% overall) of a white solid mp 220-221°C (turns orange and bubbles). NMR (D_2O) (60 MHz): 0.8-2.1 (m, 14H); 3.3 (m, 2H); 7.0 (d, J=527 Hz, 1H) ppm.

EXAMPLE 3A

Methyl N-CBZ-1-Amino-2-cyclohexylethylphosphinate

A solution of 7.00 g (0.037 moles) of 1-amino-2-cyclohexylethylphosphonous acid in 105 ml of dioxane and 40 ml of 1N NaOH was cooled to 0°C in an ice bath and stirred vigorously while 10.50 ml (12.53 g; 0.073 moles) of benzyl chloroformate and 80 ml of 1N NaOH were added rapidly and simultaneously over a period of approximately 1 minute. The pH was adjusted to the 8-9 range using 1N NaOH (hydrion paper) added in small increments. The reaction mixture was allowed to come to room temperature, and

reaction mixture was allowed to come to room temperature. After a total reaction time of 17 hours, the dioxane was removed in vacuo and the aqueous was acidified with 1N $KHSO_4$. The mixture was extracted with 3 x 50 ml of ethyl acetate. The combined ethyl acetate solution was dried (anhydrous Na_2SO_4) and evaporated in vacuo to a slightly cloudy oil.

The oil was redissolved in 50 ml of ethyl acetate and treated with 50 ml of ethereal diazomethane solution. The reaction mixture was stirred at room temperature for 2 hours, then the volatiles were removed completely in vacuo to give a thick oil. The crude product was chromatographed on silica gel using 18:1:1 methylene chloride:acetone:methanol as the eluant to give 1.17 g (100%) of product as a very thick oil. NMR ($CDCl_3$) (60 MHz): 0.0-2.2 (m containing 9H s at 1.5, 23H (total)); 3.7, 3.9 (s, 3H); 5.2-6.0 (m, 1H); 6.9 (d, J=552 Hz, 1H) ppm.

EXAMPLE 5A

Methyl (N-CBZ-1-amino-2-cyclohexylethyl) 2-carbomethoxy-4-methylpentylphosphinate

A solution of 2.75 g (0.008 moles) of methyl 1-CBZ-amino-2-cyclohexylethylphosphinate in 25 ml of absolute methanol was cooled to 0°C in an ice bath and 4.60 ml of 2M NaOMe in methanol (0.009 moles) was added via syringe. The reaction mixture was stirred at 0°C for 10 minutes, at which time 1.21 g (0.009 moles) of methyl 2-(2-methylpropyl)acrylate was added all at once. The reaction was allowed to proceed at 0°C for 30 minutes, th n th ice bath was removed and

crude product was purified by silica gel chromatography using ethyl acetate as an eluant to afford 4.77 g (61%) of the product as a very viscous oil. NMR ($CDCl_3$) (60 MHz): 0.9 (m, 6H); 0.8-2.2 (m, containing 9H s at 1.5, 29H total); 3.6, 3.8 (s, 3H); 3.7 (s, 3H) ppm.

EXAMPLE 7A

Methyl (1-amino-2-cyclohexylethyl) 2-carbomethoxy-4-methylpentylphosphinate

A mixture of 1.86 g (0.004 moles) of the ester product of Example 5A and 0.95 g of 10% Pd on carbon in 30 ml of absolute methanol was hydrogenated on a Parr type apparatus at 40 psig of hydrogen for 20 hours. The reaction mixture was filtered through a small pad of celite and the pad washed well with methanol. The filtrate was evaporated completely in vacuo to afford the pure free amine (1.34 g; 100%) as a viscous oil. NMR ($CDCl_3$) (300 MHz) 0.8-1.0 (m, 6H); 0.8-3.0 (m, 20H); 3.7-4.0 (series of s, total 6H); 8.1 (very br s, 2H) ppm.

EXAMPLE 8A

Methyl (1-amino-2-cyclohexylethyl) 2-carbomethoxy-4-methylpentylphosphinate hydrochloride

A solution of 1.13 g (0.003 moles) of the product of Example 6A in 20 ml of absolute methanol was treated with 12 ml of HCl/methanol (144 g of HCl in 400 ml methanol). The reaction mixture was stirred at room temperature for 4.5 hours. Analysis by thin lay r chromatography indicated that no more starting material remained. The volatiles were removed in vacuo and replaced several times with more

EXAMPLE 10A

Methyl [N-(N^{im}-DNP-histidyl)-1-amino-2-cyclohexyl] 2-carbomethoxy-4-methylpentylphosphinate hydrochloride

A solution of 0.949 g (0.0013 moles) of the product of Example 9A in 10 ml of absolute methanol was treated with 15 ml of HCl in methanol (144 g HCl/400 ml methanol). The reaction mixture was stirred at room temperature for 1 hour. The volatiles were removed completely in vacuo to afford 0.795 g (90%) of the crude product as a brown foam. NMR ($CDCl_3$) (300 MHz): 0.8-1.0 (m, 6H); 0.9-2.4 (m, 20H); 2.9 (br s, 2H); 3.2 (m, 1H); 3.7 (br s, 6H); 7.0 (m, 1H); 7.5-7.9 (m, 2H); 8.6 (m, 1H); 8.8 (m, 1H) ppm.

EXAMPLE 11A

N-CBZ-2-Amino-3-(1-naphthyl)propionic acid

A solution of 0.700 g (0.003 moles) of 2-amino-3-(1-naphthyl)propionic acid in 10 ml of dioxane and 3.2 ml of 1N NaOH was cooled to 0°C in an ice bath and 6.40 ml of 1N NaOH and 0.923 ml (1.10 g; 0.0064 moles) of benzyl chloroformate were added simultaneously and rapidly via syringe. The reaction mixture was stirred vigorously and the pH was adjusted to the 8-9 range by adding 1N NaOH dropwise. The reaction was allowed to come to room temperature of its own accord while stirring vigorously. After 24 hours at room temperature, the dioxane was removed in vacuo and the aqueous washed with 2 x 10 ml of ether. The ether layers were discarded and the aqueous layer was acidified to pH=1-2 (hydrion pap r) using 1N $KHSO_4$. The aqueous solution was extracted with 2 x 50 ml of ethyl acetate. The combined ethyl

EXAMPLE 13A

Methyl [N-(N-(N-CBZ-2-amino-3-(1-naphthyl)propionyl)-N^{im}-DNP-histidyl)-1-amino-2-cyclohexylethyl] 2-carbomethoxy-4-methylpentylphosphinate

A mixture of 0.176 g (0.0005 moles) of N-CBZ-2-amino-3-(1'-naphthyl)-propionic acid and .156 g (0.00055 moles) of disuccinimidyl oxalate in 7 ml of dry acetonitrile was treated with 0.045 ml (0.00055 moles) of dry pyridine. The reaction mixture was stirred at room temperature under nitrogen atmosphere for 20 hours. The mixture became homogeneous during this period. At this time, a solution of 0.300 g (0.00043 moles) of the product of Example 10 in a mixture of 8 ml of dry acetonitrile and 0.28 ml (0.202 g) of triethylamine was added all at once. The reaction turned very dark and stirring at room temperature under nitrogen was continued for 20 hours. The volatiles were evaporated in vacuo and the residue added to 30 ml of ethyl acetate, which was washed with 2 x 10 ml of saturated $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, and the volatiles removed in vacuo. The residue was chromatographed on silica gel using 18:1:1 methylene chloride:acetone:methanol as the eluant to afford 0.348 g (86%) of the tripeptide as a brownish-yellow glassy foam. NMR ($CDCl_3$) (300 MHz): 0.7-0.9 (m, 6H); 0.9-2.3 (m, 20H); 2.8-3.5 (m, 3H); 3.5-3.8 (series of singlets, total=6H); 4.1-4.8 (m, 2H); 5.0 (s, 2H); 5.3-5.6 (m, 1H); 6.8-7.6 (m, 14H); 7.8 (t, 1H); 7.9 (t, 1H); 8.2 (d, 1H); 8.5 (br, t, 1H); 8.8 (s, 1H) ppm.

EXAMPLE 15A

Methyl [N-(N-(N-CBZ-2-amino-3-(1-naphthyl)propionyl)-histidyl-1-amino-2-cyclohexylethyl] 2-carbomethoxy-4-methylpentylphosphinate

A solution of 0.100 g (0.0001 moles) of the product of Example 13 in 4 ml of dry DMF was put in a Fisher-Porter tube and approximately 20 ml of ammonia was condensed into the tube also. The reaction turned dark purple. The tube was sealed and stirring was continued for approximately 3 hours. During this time the color went from purple to pink. The tube was opened and the ammonia was allowed to evaporate. The remaining solution was transferred to a flask and the volatiles were removed completely in vacuo. The residue was chromatographed on silica gel, giving 0.018 g (22%) of the desired product as a light yellow oil. NMR ($CDCl_3$) (300 MHz): 0.8-1.0 (m, 6H); 1.0-1.8 (m, 23H); 1.9-3.4 (m, 2H); 2.8-3.1 (m, 1H); 3.4 (m, 1H); 3.7-3.8 (series of singlets, total=6H); 4.6 (m, 1H); 5.1 (s, 1H); 5.2 (br s, 2H); 5.6 (m, 1H); 7.2-7.7 (m, 11H); 7.8 (d, 1H); 7.9 (d, 1H); 8.3 (br d, 1H) ppm.

EXAMPLE 16A

[N-(N-(N-CBZ-2-amino-3-(1-naphthyl)propionyl)histidyl-1-amino-2-cyclohexylethyl] 2-carboxy-4-methylpentyl-phosphinic acid disodium salt

A solution of 0.018 g (0.000022 moles) of the product of Example 15A in 1 ml of ethanol was treated with 0.44 ml of 0.10N NaOH solution (0.000044 moles). The reaction mixture was stirred at room temperature for 16 hours. The volatiles were vaporated completely in vacuo and the residue

again immediately thereafter. Heating at 100°C was continued for a period of 4 hours. At this time, the reaction mixture was cooled to 0°C in an ice bath, and the solid filtered off and washed with 3 x 10 ml portions of ice-cold ethanol. The solid was dried in vacuo to afford 11.00 g of solid product as a fluffy white powder.

The crude powder was dissolved in 120 ml of trifluoroacetic acid, and the resulting solution was heated to 100°C in an oil bath. After approximately 5 minutes, the reaction had turned dark purple. Heating was continued for 1 hour. The reaction mixture was cooled to room temperature and the residue was partitioned between 250 ml of water and 100 ml of ether. The ether layer was separated and the aqueous washed with another 100 ml of ether. The ether was discarded and the aqueous solution evaporated completely in vacuo. The remaining white solid was treated with 10 x 50 ml of methanol, each time evaporating the methanol on a rotary evaporator. The white solid that remained was triturated with 25 ml of anhydrous ether to afford 3.77 g (36% overall) of the desired product. NMR (D_2O) (60 MHz): 1.0-2.4 (m, 9H); 3.2 (m, 1H); 5.4-5.8 (m, 2H); 6.9 (d, J=528Hz, 1H) ppm.

EXAMPLE 20A

Methyl N-CBZ-1-amino-2-(1,2,3,6-tetrahydrophenyl)-ethylphosphinate

A solution of 2.50 g (0.013 moles) of 1-amino-2-(1,2,3,6-tetrahydrophenyl)ethylphosphonous acid in 15 ml of 1N NaOH and 30 ml of dioxane that had been cooled to 0°C in an ic bath was stirred

acid in 16 ml of dioxane and 16 ml of 0.5N NaOH was cooled to 0°C in an ice bath, whereupon 2.00 ml (1.90 g; 0.0087 moles) of di-tert-butyl dicarbonate was added all at once. The reaction mixture was stirred vigorously at 0°C for 5 minutes, then removed from the ice bath and stirred at room temperature for 3 hours. The dioxane was removed in vacuo and the aqueous solution acidified to pH=1-2 (hydrion paper) with 1N $KHSO_4$. The mixture was extracted with 2 x 75 ml of ethyl acetate. The combined ethyl acetate was dried over anhydrous Na_2SO_4, filtered, and the volatiles evaporated in vacuo to give a viscous oil.

The residual oil was dissolved in 50 ml of ethyl acetate and treated with 40 ml of ethereal diazomethane solution. The reaction mixture was stirred at room temperature for 1 hour and 10 minutes, then the volatiles were evaporated completely in vacuo. The residual oil was chromatographed on silica gel using 25:1:1 methylene chloride:acetone: methanol to afford 1.83 g (95%) of the product as a very thick yellow oil that crystallized very slowly. NMR ($CDCl_3$) (60 MHz): 1.0-2.2 (m containing 9H s at 1.4, total=19H); 3.7, 3.9 (singlets, total=3H); 3.9-4.2 (m, 1H); 5.0-5.7 (m, 2H) ppm.

EXAMPLE 22A

Methyl [N-BOC-1-amino-2-(1,2,3,6-tetrahydro)phenyl-ethyl] 2-carbomethoxy-4-methylpentylphosphinate

A solution of 0.700 g (0.003 moles) of the product of Example 21A in 5 ml of absolute methanol was cooled to 0°C whereupon 1.65 ml (0.0033 moles) of 2N NaOMe in methanol was added over a on minut

EXAMPLE 24A

Methyl [N-(N-BOC-glycylglycyl)-1-amino-2-(1,2,3,6-tetrahydro)phenylethyl] 2-carbomethoxy-4-methylpentyl-phosphinate

A suspension of 0.202 g (0.00087 moles) of N-BOC-glycylglycine and 0.272 g (0.00096 moles) of disuccinimidyl oxalate in 13 ml of dry acetonitrile was treated with 0.078 ml (0.076 g; 0.00096 moles) of dry pyridine. The heterogeneous mixture was stirred at room temperature under nitrogen atmosphere and slowly became homogeneous over a period of several hours. The reaction was stirred at room temperature for a total for 17 hours. At this time, a solution of the product of Example 23 in 14 ml of acetonitrile containing 0.484 ml (0.352 g; 0.0035 moles) of triethylamine was added all in one portion. The reaction mixture was allowed to stir at room temperature under nitrogen atmosphere for 48 hours. At this time the volatiles were evaporated in vacuo and the residue dissolved in 30 ml of ethyl acetate. The organic solution was washed with 2 x 20 ml of saturated $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, and the volatiles removed in vacuo. The residue was purified by silica gel chromatography using 18:1:1 methylene chloride:acetone:methanol as the eluant to afford the product as a light yellow oil. NMR ($CDCl_3$) (300 MHz): 0.8-1.0 (m, 6H); 1.1-1.9 (m containing 9H s at 1.5, total=22H); 1.9 (br s, 1H); 2.2 (m, 1H); 2.9 (m, 1H); 3.6-3.8 (series of singlets; total=6H); 3.7-4.2 (m, 4H); 4.5 (m, 1H); 5.3-5.8 (m, 2H); 6.7-7.2 (m, 2H) ppm.

was treated with HCl gas for 45 minutes. The reaction was allowed to stand overnight and then filtered to remove a small amount of solid material. The remaining 29.15 g (0.084 moles) of starting material was dissolved in 430 ml of anhydrous ether and treated similarly. The combined filtrates from the two reactions was evaporated in vacuo and the viscous oil treated with several 200 ml portions of carbon tetrachloride followed by evaporation in vacuo. This procedure led to a sticky crystalline mass which was triturated with anhydrous ethyl ether and filtered and then vacuum dried to afford 41.55 g (64%) of the salt as a white crystalline solid. NMR ($CDCl_3$) (60 MHz): 1.0-1.6 (m, 6H); 2.1 (s, 2H); 3.2-4.5 (m, 9H); 7.2 (s, 10H) ppm.

EXAMPLE 27A

Diethyl 2-Amino-3-phenylethylphosphonate hydrochloride

A solution of 2.00 g (0.0052 moles) of the product of Example 26A in 8 ml of absolute ethanol containing 0.200 g of 10% Pd on carbon was hydrogenated in a Parr type apparatus for 7 hours at 40 psig of hydrogen. The reaction mixture was filtered through a celite pad and the pad washed well with ethanol. The combined filtrates were evaporated in vacuo to give 1.50 g (99%) of the product as a very viscous, colorless oil. NMR (d_2-acetone) (60 MHz): 1.0-1.6 (overlapping t, 6H); 3.3-5.0 (m, 7H); 7.0-7.6 (m, 5H) ppm.

cyclohexylacetaldehyde (7.2 g, 0.057 mol). After stirring for 30 minutes at 0°C and 2 hours at room temperature, the reaction was filtered and the solvents removed by evaporation in vacuo. The residue was redissolved in 100 ml of dichloromethane cooled to 0°C and esterified with an ether solution of diazomethane. The solvents and excess diazomethane were subsequently removed by evaporation in vacuo and the crude product was purified by chromatography to give 11 g of the title compound.

Chromatography: silica, ethyl acetate

TLC (silica, ethyl acetate) R_f=0.48

NMR ($CDCl_3$, TMS) 0.9-2.0 (m, 13H), 2.95 (d 16Hz, 2H), 3.64 (s, 3H), 3.7 (d 12Hz, 3H), 3.9-4.5 (m.1H), 5.1 (s,2H), 5.4 and 5.8 (d 10Hz, 1H), 7.2 (s.5H)

mass spectrum: M^+

EXAMPLE 31A

Methyl (N-CBZ-1-amino-3-methylbutyl) carbomethoxymethylphosphinate

This ester is prepared by the procedure described in Example 33A, using 3-methylbutyraldehyde in place of cyclohexylacetaldehyde. It can also be prepared by the method of P. A. Bartlett et al., J. Amer. Chem. Soc., 106, 4282-83 (1984).

EXAMPLE 32A

N-CBZ-1-aminoethylphosphinic acid

The title compound was prepared using the procedure described in Example 2A with paraldehyde replacing hexahydrophenylacetaldehyde.

products were isolated; fraction A (0.190 g, 0.43 mmol, 26%) and fraction B (0.217 g, 0.49 mmol, 30%). Product A, which eluted from the column first, was identified as the E isomer by analysis of its 300 MHz proton NMR spectrum (olefinic proton resonance at 6.68 ppm ($CDCl_3$). Product B is the Z isomer (olefinic proton resonance at 6.03 ppm).

Anal. Calcd for $C_{22}H_{40}NO_6P$:
C, 59.31; H, 9.05; N, 3.14.

Found: (Fraction A) C, 59.07; H, 8.86; N, 3.08;
(Fraction B) C, 59.47; H, 8.82; N, 3.15.

MS (FAB) 446 (both isomers) (M^+ +1).

EXAMPLE 34A

Methyl 2-(2-methylpropyl)acrylate

This compound was prepared by the method of J. Harley-Mason et al. (Tetrahedron 1980, 36, 1063) in approximately 35% overall yield. NMR ($CDCl_3$) (300 MHz): 0.9 (d, 6H); 1.8 (septet, 1H); 2.2 (d, 2H); 3.7 (s, 3H); 5.5 (d, 1H); 6.1 (d, 1H) ppm.

EXAMPLE 35A

Methyl 2-(cyclohexylmethyl)acrylate

This compound was prepared as above in approximately 20% overall yield from dimethyl malonate and bromomethylcyclohexane. NMR ($CDCl_3$) (60 MHz): 0.8-2.0 (m, 11H); 2.2 (d, 2H); 3.7 (s, 3H); 5.4 (m, 1H); 6.0 (d, J=2Hz, 1H) ppm.

EXAMPLE 36A

Methyl 2-(n-propyl)acrylate

This compound was prepared as above in approximately 45% overall yield from dimethyl

crystalline solid. 28H); 2.7-3.2 (m, 6H); 3.5-3.8 (m, 6H); 4.1-5.4 (m, 3H); 6.8-7.6 (m, 6H); 7.6-7.9 (m, 2H); 8.0 (br s, 1H) ppm.

EXAMPLE 39A

Methyl (N-CBZ-1-amino-2-cyclohexyl)2-carbomethoxy-3-cyclohexylpropylphosphinate

A solution of 3.39 g (0.010 moles) of the product of Example 3A in 25 ml of absolute methanol was cooled to 0C under N_2. At this time, a solution of 5.50 ml of 2N NaOMe in methanol was added dropwise over a period of ca. 1 min. The reaction mixture was stirred for 10 min. and at this time, 1.91 g (0.0105 moles) of the product of Example 35 was added over a 1 min. period. The reaction mixture was stirred at 0°C for 30 min., then at room temperature for 23 hours. The volatiles were removed in vacuo and the residue added to 50 ml of ice-cold 1N HCl. The mixture was extracted with 3 X 50 ml of ethyl acetate. The combined ethyl acetate was dried over anh. $MgSO_4$, filtered, and the volatiles evaporated in vacuo to a colorless oil. The residue was chromatographed on silica gel using 18:1:1 methylene chloride:acetone:methanol as the eluant to afford 4.26 g (82%) of the desired product as a very viscous oil. NMR ($CDCl_3$) (300 MHz): 0.7-1.9 (m, 28H); 2.1 (m, 1H); 2.9 (br s, 1H); 3.6-3.8 (m, 6H); 4.1 (br s, 1H); 5.1 (s, 2H); 7.3 (s, 5H) ppm.

EXAMPLE 40A

Methyl (1-amino-2-cyclohexyl)2-carbomethoxy-3-cyclohexylpropylphosphinate

A mixture of 4.00 g (7.7 mmol) of the product f Example 39 in 50 ml of absolute methanol

volatiles evaporated in vacuo. The residue was chromatographed on silica gel using 18:1:1 methylene chloride:acetone:methanol as the eluant to afford 2.69 g (65%) of the desired product as a viscous dark yellow oil. NMR ($CDCl_3$) (300 MHz): 0.7-1.9 (m containing 9H s at 1.4, 37H); 2.8 (br s, 1H); 3.1 (m, 1H); 3.7 (m, 6H); 4.4 (br s, 1H); 6.0 (m, 1H); 7.0 (m, 1H); 7.5-8.0 (m, 2H); 8.6 (dd, 1H); 8.9 (s, 1H) ppm.

EXAMPLE 42A

Methyl [N-(N^{im}-DNP-histidyl)-1-amino-2-cyclohexyl-ethyl] 2-carbomethoxy-3-cyclo-hexylpropylphosphinate

A solution of 2.00 g (2.6 mmol) of the product of Example 41A in 20 ml of methanol was treated all at once with 20 ml of HCl in methanol (134.4 g of HCl in 400 ml of methanol). The reaction mixture was stirred at room temperature for 1 hour 15 minutes. The volatiles were removed completely in vacuo, and the residue triturated with 2 X 15 ml of anhydrous ether to afford 1.83 g (99%) of the desired product. NMR ($CDCl_3$) (300 MHz): 0.7-2.0 (m, 28H); 2.2 (m, 1H); 2.8 (m, 1H); 3.4-3.9 (m, 6H); 4.3 (br s, 1H); 4.8 (br s, 1H); 7.4-9.6 (m, 8H) ppm.

EXAMPLE 43A

Methyl [N-(N-(N-CBZ-2-amino-3-(1-naphthyl)propionyl)-N^{im}-DNP-histidyl)-1-amino-2-cyclohexylethyl]2-carbomethoxy-3-cyclohexylpropylphosphinate

A mixture of 0.353 g (1.00 mmol) of the product of Example 11A and 0.312 g (1.1 mmol) of disuccinimidyl oxalate in 10 ml of dry acetonitrile was treated with 0.087 g (0.09 ml; 1.1 mmol) of

homogeneous. At this time, a mixture of 0.727 g (1.00 mmol) of the product of Example 42 and 0.404 g (4.00 mmol; 0.56 ml10 of triethylamine in 10 ml of acetonitrile was added all at once. The reaction mixture turned darker and stirring at room temperature under N_2 was continued for 24 hours. At this time, the volatiles were removed in vacuo and the residue dissolved in 50 ml of methylene chloride. The organic solution was washed with 2 X 50 ml of saturated $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, and the volatiles removed in vacuo. The residue was chromatographed on silica gel using 18:1:1 methylene chloride:acetone:methanol as the eluant to give 0.571 g (58%) of the desired product as a glassy foam. NMR ($CDCl_3$) (300 MHz)-: 0.7-1.9 (m, containing 9H s at 1.2, 37H); 2.7-3.3 (m, 4H); 3.5-3.8 (m, 6H); 4.4 (m, 3H); 4.7 (m, 1H); 5.0 (m, 1H); 6.8-8.0 (m, 9H); 8.2 (d, 2H); 8.6 (dd, 2H); 8.8 (t, 1H) ppm.

EXAMPLE 45A

Methyl [N-(N-(N-CBZ-2-amino-4-phenylbutyryl)-N^{im}-DNP-histidyl)-1-amino-2-cyclo-hexylethyl] 2-carbomethoxy-4-methylpentylphosphinate

A mixture of 0.313 g (1.00 mmol) of N-CBZ-2-amino-4-phenylbutyric acid and 0.312 g (1.1 mmol) of disuccinimidyl oxalate in 20 ml of dry acetonitrile was treated with 0.087 g (1.1 mmol; 0.90 ml) of pyridine. The reaction mixture was stirred at room temperature under N_2 for 24 hours. During this period the reaction went from heterogeneous to homogeneous. At this time a mixture of 0.687 g (0.001 moles) of the product of Example 10 and 404 mg

stirred at room temperature under N_2 for 30 hours. The volatiles were removed in vacuo and the residue was dissolved in 50 ml of methylene chloride. The organic mixture was washed with 2 X 15 ml of saturated $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, and the volatiles evaporated in vacuo. The residue was chromatographed on silica gel using 18:1:1 methylene chloride:acetone:methanol as the eluant. This provided 0.493 g (54%) of the desired product as a glassy foam. NMR ($CDCl_3$) (300 MHz): 0.7-1.0 (m, 6H); 1.0-2.2 (m containing 9H s at 1.5, 28H), 2.6 (m, 2H); 2.7-3.3 (m, 3H); 3.5-3.8 (m, 8H); 4.0 (m, 2H); 4.4 (m, 1H); 4.7 (m, 1H); 5.0 (m, 1H); 6.9-8.0 (m, 7H); 8.5 (m, 2H); 8.8 (m, 1H) ppm.

EXAMPLE 47A

Methyl [N-(N-(N-CBZ-phenylalanyl)-N^{im}-DNP-histidyl)-1-amino-2-cyclohexylethyl]-2-carbomethoxy-4-methylpentylphosphinate

A mixture of 0.156 g (0.55 mmol) of disuccinimidyl oxalate and 0.150 g (0.0005 moles) of N-CBZ-phenylalanine in 10 ml of dry acetonitrile was treated with 0.04 ml (0.5 mmol) of pyridine. The reaction mixture was stirred at room temperature under N_2 for 2 hours. At this time, an additional 0.010 g of disuccinimidyl oxalate was added and stirring under N_2 continued for 5 hours. During this time the mixture went from heterogeneous to homogeneous. At this time, 0.330 g the product of Example 10 was added, immediately followed by 0.202 g (2.00 mmol; 0.28 ml) of triethylamine. The reaction mixture immediately turned darker. The mixture was stirred at room temperature for 18h. At this time

volatiles were evaporated completely in vacuo and the residue dissolved in 20 ml of ethyl acetate. The organic solution was washed with 3 X 15 ml of saturated $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered and the ethyl acetate evaporated in vacuo. The residue was chromatographed on silica gel using 18:1:1 methylene chloride:acetone:methanol as the eluant to afford 0.266 g (61%) of the desired product as a glassy foam. NMR ($CDCl_3$) (300 MHz): 0.7-0.9 (m, 6H); 0.9-2.0 (m containing 3H s at 1.3, 26H); 2.5-3.5 (m, 5H); 3.5-3.7 (m, 6H); 4.0 (br s, 1H); 4.3 (br s, 2H); 4.7 (br s, 1H); 5.0 (m, 1H); 6.8-7.0 (m, 2H); 7.0-7.3 (m, 5H); 7.5-8.0 (m, 2H); 8.6 (m, 2H); 8.9 (m, 1H) ppm.

EXAMPLE 49A

[N-(N-2-Amino-3-(1-naphthyl)propionyl-histidyl)-1-amino-2-cyclohexylethyl] 2-carboxy-4-methylpentyl-phosphinic acid

A solution of 0.045 g (0.055 mmol) of the product of Example 13A in 3 ml of ethanol was treated with 1.10 ml of 0.100N NaOH (aq.). The reaction mixture was stirred at room temperature for 20 hours, at which time an additional 1.00 ml of 0.100N NaOH was added. Stirring was continued for 1 hour, then the volatiles were evaporated completely in vacuo. The residue was triturated with 3 X 2 ml of anhydrous ether, and the residue was purified by passing it through a column containing ca. 15 g of DOWEX 50W-X4 hydrogen form ion-exchange resin (5.2 meq/dry gram). This afforded 0.007 g (15%) of the desired product as a very light yellow glassy foam. NMR (CD_3OD) (300 MHz): 0.6-0.9 (m, 6H); 1.1-2.0 (m, 20H); 2.7 (m, 2H);

own accord. The remaining volatiles were evaporated completely in vacuo and the residue triturated with anhydrous ether until a free flowing crystalline solid was obtained.

EXAMPLE 52A

Methyl [N-(N-(N-CBZ-2-amino-4-phenyl)butyryl-histidyl)-1-amino-2-cyclohexyl-ethyl] 2-carbomethoxy-4-methylpentylphosphinate

A solution of 0.200 g (0.21 mmol) of the product of Example 45A in 2 ml of dry dimethylformamide was treated with ca. 12 ml of anhydrous ammonia in a Fisher-Porter Tube. The tube was sealed and the reaction mixture stirred at room temperature for 21 hours. During this period the color of the reaction went from dark purple to reddish-brown. At this time the tube was opened and the ammonia allowed to evaporate of its own accord. The remaining volatiles were evaporated completely in vacuo and the residue was triturated with anhydrous ether until a free flowing solid was obtained.

EXAMPLE 53A

Methyl [N-(N-(N-BOC-2-amino-4-phenyl)butyryl-histidyl)-1-amino-2-cyclohexylethyl] 2-carbomethoxy-4-methylpentylphosphinate

A solution of 0.200 g (0.22 mmol) of the product of Example 46A in 2 ml of dry methylformamide was treated with ca. 12 ml of anhydrous ammonia in a Fisher-Porter Tube. The tube was sealed and the reaction mixture stirred at room temperature for 21 hours. During this period the color of the reaction went from dark purple to reddish-brown. At this time

remaining volatiles were evaporated completely in vacuo and the residue triturated with anhydrous ether. This afforded the desired product as a yellow solid after purification by chromatography on DOWEX 50W-X4.

EXAMPLE 56A

N-Methyl 1-amino-2-cyclohexylphosphinic acid

This acid is prepared using the procedure described in Example 2A with methylamine hydrochloride replacing aminophenylmethane hydrochloride.

EXAMPLE 57A

Methyl N-CBZ-N-methyl 1-amino-2-cyclohexylethyl-phosphinate

This ester is prepared from N-methyl-1-amino-2-cyclohexylphosphinic acid using the procedure described in Example 3A.

EXAMPLE 58A

Diethyl N-methyl-1-amino-2-phenylethylphosphonate

This ester is prepared using the procedure described in Example 25A with equimolar amounts of methyl amine hydrochloride and triethylamine substituted for benzylamine.

EXAMPLE 59A

Diethyl N-CBZ-N-methyl-1-amino-2-cyclohexylethyl-phosphonate

This ester is prepared by treatment of the product of Example 58A with benzyl chloroformate and Et_3N in CH_2Cl_2 and is purified by silica gel chromatography.

EXAMPLE 62A

(1-Amino-2-cyclohexylethyl) 2-carbomethoxy-4-methylpentylphosphinic acid

To the product of Example 8 (60 mg; 0.158 mmol) was added HBr-HOAc solution (5 equivalents) and an equivalent volume of HOAc. The solution was stirred for 24 hours and then concentrated to dryness, giving the product (40 mg; 61%). MS: m/e 334 (M^+ +1). NMR (D_2O): 0.6-2.3 (22H, m); 2.8 (1H, br s); 3.7 (3H, m); 3.3-3.6 (1H, m); 4.2 (1H, m); 8.0 (3H, br s).

EXAMPLE 63A

Methyl [N-(N-t-butoxycarbonyl-phenylalanyl)-1-amino-2-cyclohexylethyl] 2-carbomethoxy-4-methylpentylphosphinate

The product of Example 8A (0.26 g) was dissolved in dry CH_2Cl_2 (3 ml) and neutralized with Et_3N (0.095 ml) at 0°C. To the above solution were added sequentially N-Boc-phenylalanine (0.22 g), HOBT (0.18 g) and DCC (0.19 g). The mixture after stirring for 4 hours at 0°C was stirred at 25°C for an additional 15 hours. The mixture was filtered, and the CH_2Cl_2 phase was washed with $NaHCO_3$ (saturated), water, saturated NaCl and dried ($MgSO_4$). Removal of solvent in vacuo gave a foam which was purified by medium pressure liquid chromatography (mplc) using ethyl acetate-hexane (3:1) on a silica gel column yield 0.28 g (70%) (foam). NMR ($CDCl_3$): 7.23 (5H, s), 6.62 (1H, m), 5.15 (1H, m), 4.1-4.41 (2H, m), 3.63 (6H, m), 3.11 (2H, m), 2.85 (2H, m), 0.85-2.05 (32H, m). MS (FAB): m/e 595 (M^+ +1).

EXAMPLE 66A

[N-(Lysyl)-1-amino-2-cyclohexylethyl] 2- carboxy-4-methylpentylphosphinic acid

The product of Example 65A (60 mg) was stirred with aqueous 1N NaOH (0.18 ml) in acetone (1 ml) for 24 hours. Removal of acetone in vacuo and acidification with cold 1N HCl gave the free acid as an oil. The oil was then treated with HBr-AcOH (33%) (1 ml) for 12 hours at 25°C. Excess HBr was removed in vacuo, and the product was precipitated with dry ether. The hygroscopic solid was filtered and dissolved in MeOH (1 ml), and treated with propylene oxide (0.2 ml). The product was finally precipitated with dry ether. The solid was filtered, washed with dry ether and dried to give white powder (39 mg). NMR (CD_3OD): 4.18 (2H, m), 2.85 (2H, m), 0.85-2.15 (29H, m). MS (FAB): m/e 498 (M^+ +1).

EXAMPLE 67A

Methyl [N-(Nα-(N-BOC-2-amino-3-(1-naphthyl)propionyl)-N-CBZ-lysyl)-1-amino-2-cyclohexylethyl] 2-carbomethoxy-4-methylpentylphosphinate

The product of Example 65A (0.25 g) was dissolved in 50% TFA in CH_2Cl_2 (2 ml), and the mixture was stirred at 25°C for 1-1/2 hours. Removal of excess reagent and CH_2Cl_2 in vacuo gave an oil which upon drying over P_2O_5 and NaOH in vacuo gave the amine salt as a foam.

The above foam was dissolved in EtOAc (10 ml) and neutralized with saturated $NaHCO_3$ (aqueous). The EtOAc phase was washed with brine and dried over (Na_2SO_4). Removal of solvent in vacuo gave the free amine as an oil. The free amine was then coupled

hours at 40 psi. The catalyst was filtered off and the filtrate upon evaporation in vacuo gave the pure product as the acetate salt (40 mg). NMR ($CDCl_3$): 6.95-8.1 (11H, m), 5.60 (1H, d), 5.22 (1H, m), 4.51 (2H, m), 4.12 (1H, m), 3.68 (6H, m), 2.6-2.95 (4H, m), 2.05 (3H, s), 0.85-2.15 (39H, m). MS (FAB): m/e 773 (M^+ +1).

EXAMPLE 70A

Methyl (1-amino-2-cyclohexylethyl)carbomethoxymethyl-phosphinate hydrochloride

The product of Example 30A (3.58 g) was hydrogenated in methanol (30 ml) [containing concentrated HCl (0.72 ml)] over Pd-C (10%) (0.35 g) at 40 psi overnight at 25°C. The catalyst was filtered off and the filtrate was evaporated to give the product as glass-like solid foam (2.67 g) (94%). MS (FAB): m/e 278 (M^+ +1).

EXAMPLE 71A

Methyl [(N-BOC-phenylalanyl)-1-amino-2-cyclohexyl-ethyl] carbomethoxymethylphosphinate

The product of Example 70A (0.78 g) was treated with Et_3N (0.35 ml) in a mixture of THF-CH_2Cl_2 (1:1) (6 ml) at 0°C. The free amine, thus obtained, was coupled with N-BOC-phenylalanine (0.66 g) in presence of DCC (0.56 g) and HOBT (0.5 g) under standard condition. Filtration and processing of the filtrate gave the crude product as a foam which was purified by flash chromatography on silica gel using 10% hexane in ethyl acetate. Yield (1.18 g) (91%) (foam). MS: M+H = 525, M-99 = 425 (-BOC). ^1H NMR: 7.35-7.15 (5H, m), 5.15 (1H, br s), 4.90

(2H, s), 4.6 (1H, m), 4.05 (1H, m), 3.85-3.60 (6H, m), 3.2 (2H, brs), (2H, d, J=10), 1.90-.8 (19H, m), 1.60, 1.40 (9H, s). MS (FAB): m/e 640 (M^+ +1).

EXAMPLE 74A

Methyl [N-(Nα-BOC-lysyl)-1-amino-2-cyclohexylethyl] carbomethoxymethylphosphinate

The product of Example 73A (60 mg) was hydrogenated over Pd/C (10%) in MeOH (5 ml) containing 1 equivalent of acetic acid under standard conditions to give the titled compound as a foam (51 mg). NMR ($CDCl_3$): 8.5 (3H, br s), 8.2-7.6 (1H, m), 5.9-5.5 (1H, m), 4.5 (1H, m), 4.1 (1H, br s), 3.7 (6H, m), 3.2-2.8 (4H, m), 2.0 (3H, s), 1.90-.7 (19H, m), 1.4 (9H, s). MS: m/e 506 (M^+ +1).

EXAMPLE 75A

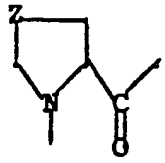
Methyl [N-(N-(2-naphthyloxy)acetyl-phenylalanyl)-1-amino-2-cyclohexylethyl] carbomethoxymethylphosphinate

The product of Example 71A (0.395 g) was treated with trifluoroacetic acid -CH_2Cl_2 (1:1) (5 ml) at 25°C for 2 hours. Removal of excess reagent in vacuo gave the trifluoroacetate salt of the free amine as a foam, which was dissolved in dry THF (5 ml) and neutralized with Et_3N (0.12 ml). To the free amine, thus obtained, (2-naphthoxy)-acetic acid N-hydroxysuccinimide ester (0.31 g) was added, and the mixture was stirred at 25°C for 24 hours. The crude product obtained, after removal of solvent in vacuo, was purified by mplc using 25% hexane in ethyl acetate. Yield 0.34 g (65%) (foam). MS: M+H = 609. ^1H NMR: 7.8-7.0 (12H, m), 6.9 (1H,

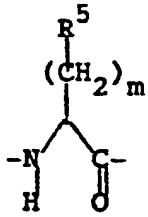
the corresponding trifluoroacetate salt of the amine was obtained as described before. The salt was dissolved in dry THF (4 ml), neutralized with Et_3N (0.05 ml) and coupled with N-CBZ-ß-naphthylalanine (0.125 g) in the presence of HOBT (0.072 g) and DCC (0.081 g) under standard conditions. The reaction was filtered, and the filtrate was washed with saturated $NaHCO_3$, water and dried ($MgSO_4$). Removal of the solvent gave an oil which was purified by mplc using 20% hexane in ethyl acetate. Yield 0.21 (68%). ^1H NMR: 8.3-7.1 (17H, m), 7.0-5.2 (4H, m), 5.1 (4H, m), 4.4-4.0 (2H, m), 4.5 (2H, m), 3.8-3.4 (6H, m), 3.1-2.8 (4H, m), 1.9-.6 (19H, m).

Claims to the invention follow.

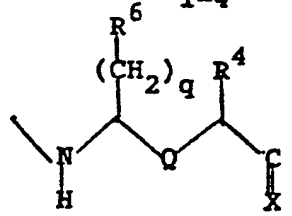
where R^1 is as defined further below;

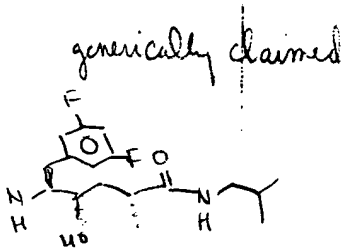
D is absent; or  , where Z is

$-(CH_2)_1-$ and 1 is 1 or 2; or -S-;

E is absent; or  , where m is 1 to 4; and

R^5 is hydrogen; C_{1-4} alkyl; aryl; aryl-C_{1-4} alkyl; aryl C_{1-4} alkyl or aryl where the aryl portion is substituted with up to three members selected from the group consisting of C_{1-4} alkyl, trifluoromethyl, hydroxy, C_{1-4} alkoxy, and halo; or indolyl;

G is (1)  . where q is 1 to 4; X is O, or H, H;

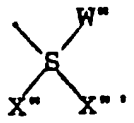


R^4 is hydrogen; or  ,

where R^9 is hydrogen; C_{1-4}alkyl; hydroxy, or C_{3-7}cycloalkyl; and

R^3 is hydrogen; C_{1-4}alkyl; aryl; aryl C_{1-4}alkyl; aryl C_{1-4}alkyl or aryl substituted with up to three members selected from the group consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, and halo; or indolyl;

and V' is -O- or -NH-; amino; or mono- or di-C_{1-4}alkyl amino; and W' is absent; -O-; -NH-; or $-CH_2-$;



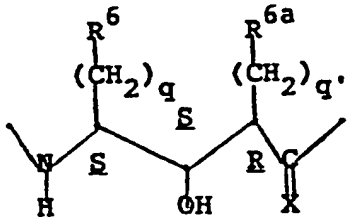
where X" and X"' are independently

absent; or  ; and

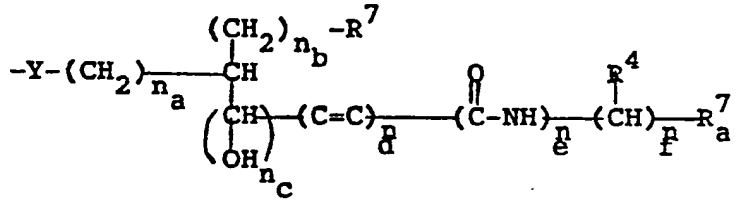
W" is absent; $-CH_2-$; or  , where R^8 is hydrogen or C_{1-3} alkyl;

 ;  ; or

 ; where R is hydrogen; C_{1-4} alkyl; formyl; C_{1-4} alkanoyl; aroyl; carboxy; C_{1-4} alkoxycarbonyl; aryloxycarbonyl; or aryl C_{1-4} alkoxycarbonyl; or

(2)  where q is 1 to 4; q' is 0 to 4; X is O or H, H;

heterocyclic C_{1-4}alkyl;
$N(R')^+_3 A^-$, where R' is as defined above, and A^- is a counterion; guanidyl; heterocyclic; heterocyclic substituted with up to five members independently selected from the group consisting of C_{1-6}alkyl, hydroxy, trifluoromethyl, C_{1-4}alkoxy, halo, aryl, aryl C_{1-4}alkyl, amino, and mono- or di-C_{1-4}alkylamino; or heterocyclic substituted with another, the same or different, heterocyclic;

(2) 

where
Y is as defined above;
n_a is 0 or 1;
n_b is 1 to 4;
n_c is 0 or 1;
n_d is 0 or 1;
n_e is 0 or 1, provided that n_e cannot be 1 when n_d is 0;
n_f is 1 to 4;

R^4 is hydrogen; or $-\underset{R^3}{CH}-R^9$, where

R^9 is hydrogen; C_{1-4}alkyl; hydroxy; or

where
n is 0 or 1; and
R^8 is as defined above; or

(b) $-(CH_2)_n-\underset{\underset{CH_2}{|}}{C}-$

where
n is 0 or 1; or

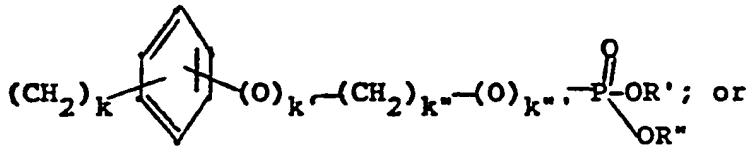
(4) (a) $Y-\underset{\underset{q}{|}}{\overset{\overset{R^{10}}{|}}{(CH)}}-R^{11}$; (b) $Y-\underset{\underset{q'}{|}}{\overset{\overset{R^{12}}{|}}{(CH)}}-R^{13}$; or

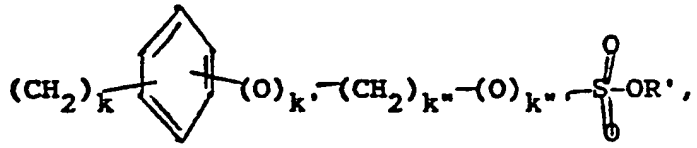
(c) $Y-\underset{\underset{R^{14}}{|}}{CH}-R^{11}$

where
Y is -NH- or -O-;
q is 1-5;
q' is 0-5;
R^{10} is hydrogen; hydroxy; $N(R'')_2$, where R'' may be the same or different and is hydrogen or C_{1-4}alkyl; guanidyl; or $\overset{+}{N}(R'')_3A^-$, where R'' is as defined above, and A^- is a counterion; provided that at least one R^{10} is not hydrogen;
R^{11} is C_{1-4}alkyl; C_{3-7}cycloalkyl; aryl; aryl substituted with up to three members independently selected from the group consisting of C_{1-6}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, amino, mono- or di- C_{1-4}alkylamino, amino

guanidyl, and guanidyl-C_{1-4}alkyl; and

R^{14} is carboxy, ester or amide;

(d) 

(e) 

where

Y is -NH- or -O-;

k is 0-4;

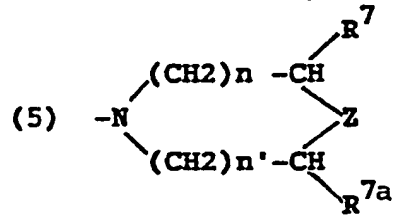
k' is 0 or 1;

k" is 0-4;

k"'is 0 or 1;

R' is hydrogen or C_{1-4}alkyl; and

R" is hydrogen or C_{1-4}alkyl;

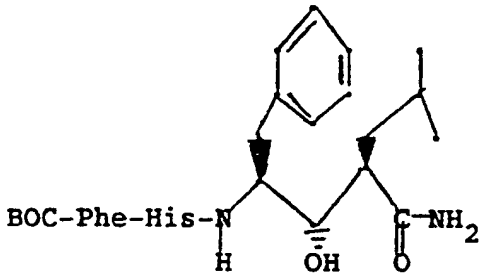
(5) 

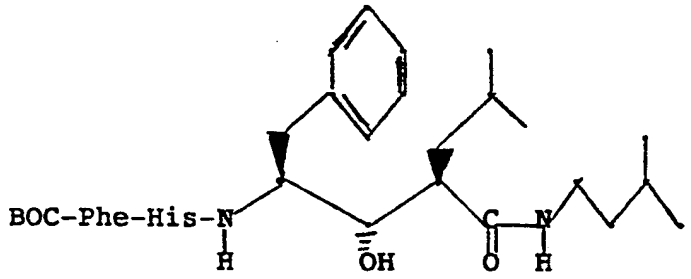
where Z is NH, N-R^7,O,S or CHR^7;

n' is 0 to 5; and

R^{7a} is hydrogen, hydroxy, C_{1-4}-alkyl,C_{3-7}-cydoalkyl, aryl,ary l substituted with from one to five members independently selected from the group consisting of C_{1-6}-alkyl trifluoromethyl, hydroxy, C_{1-4}alkoxy, amino, mono- or di- C_{1-4} alkylamino,

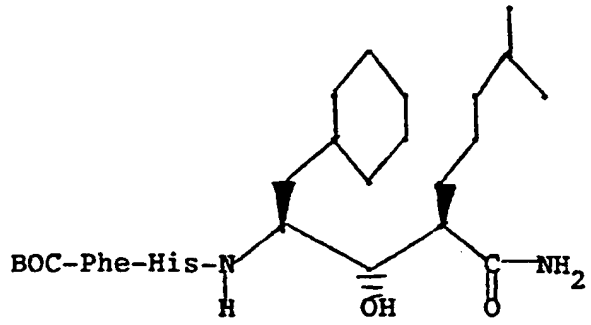
BOC-His-Pro-Phe-His-Sta-OEt

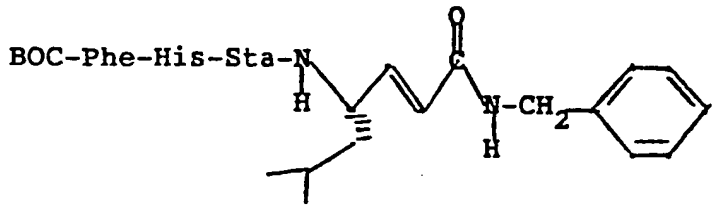


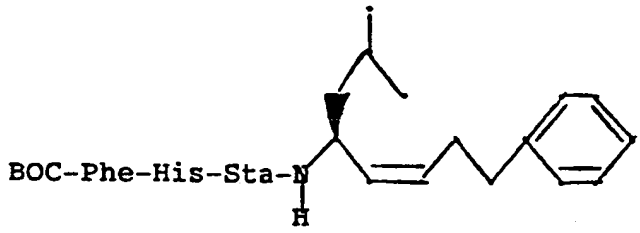


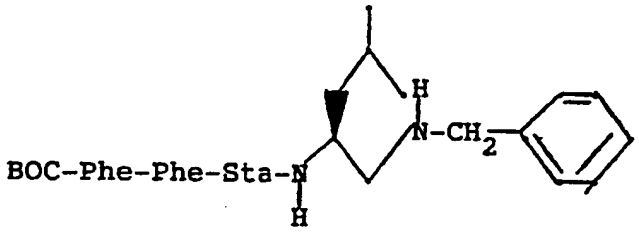
BOC-Phe-His-ACHPA-NH_2

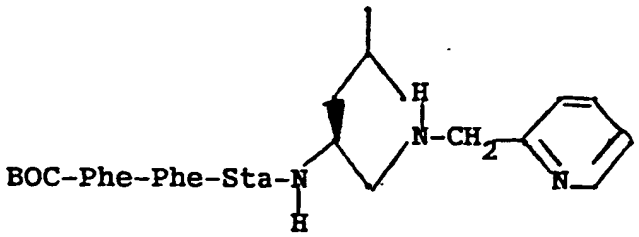
BOC-HomoPhe-His-Sta-NH_2

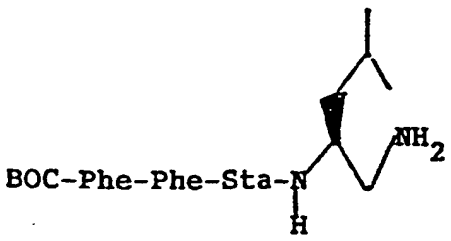


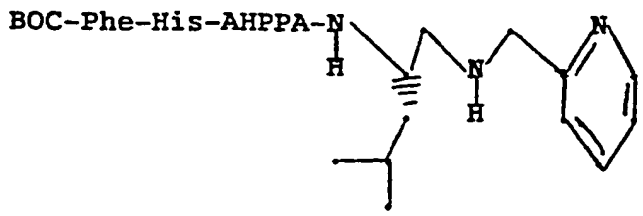

BOC-Phe-His-Sta-N
H
O
C
N-CH2
H

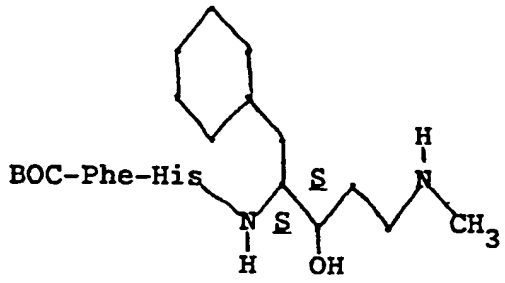

BOC-Phe-His-Sta-N
H

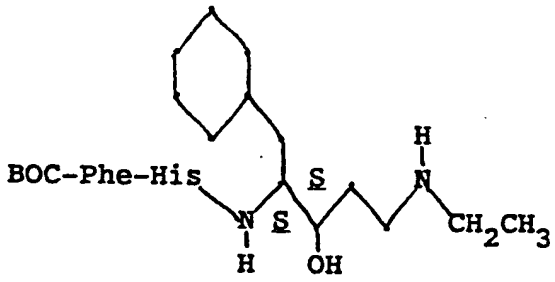

H
N-CH2
BOC-Phe-Phe-Sta-N
H

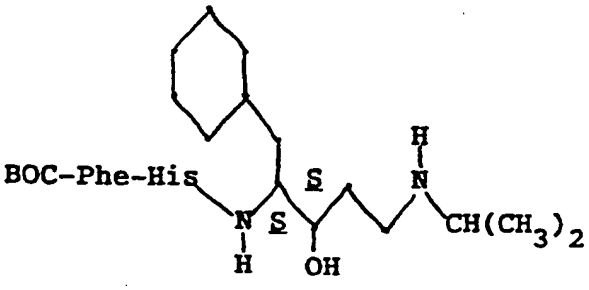

H
N-CH2
BOC-Phe-Phe-Sta-N
H
N

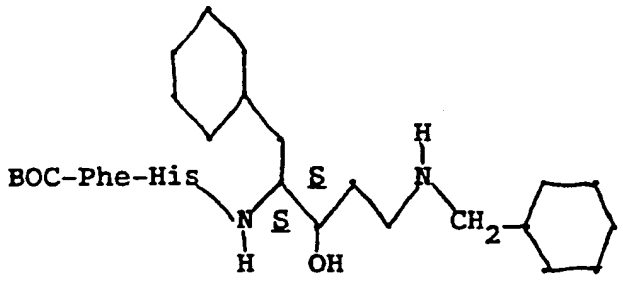

NH2
BOC-Phe-Phe-Sta-N
H

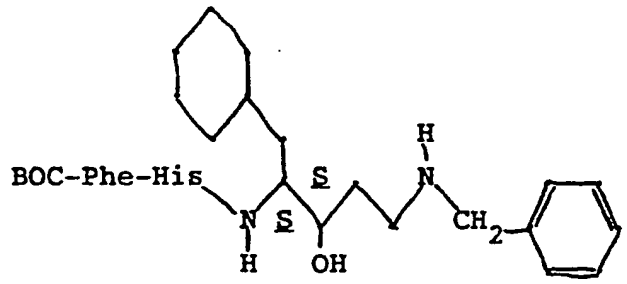

BOC-Phe-His-AHPPA-N
H
N
H
N

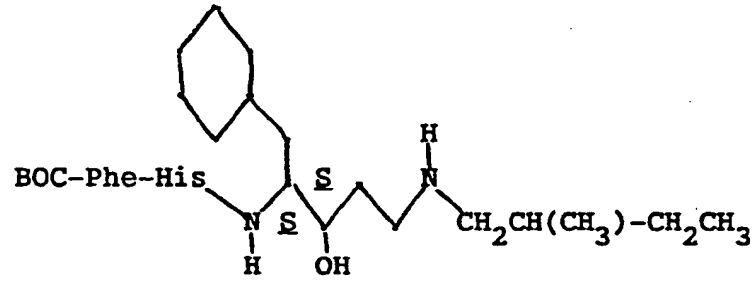

BOC-Phe-His
S
N
S
H
OH
H
N
CH3

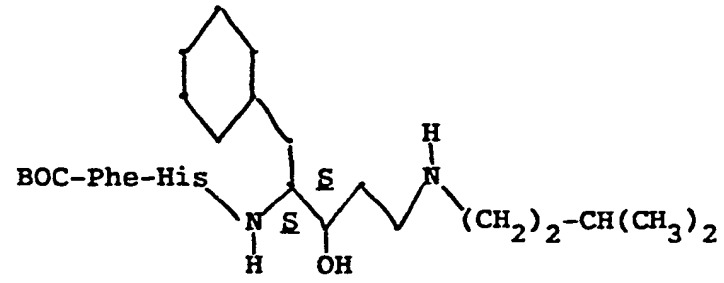

BOC-Phe-His
S
N
S
H
OH
H
N
CH2CH3

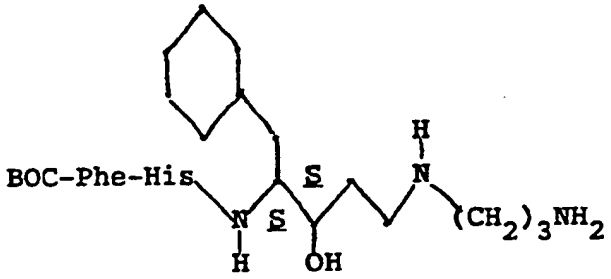

BOC-Phe-His
S
N
S
H
OH
H
N
CH(CH3)2

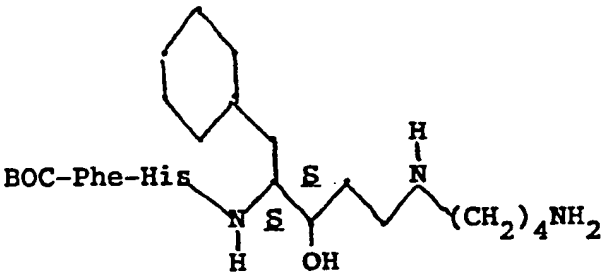

BOC-Phe-His
H
N
S
N
S
CH2
H
OH

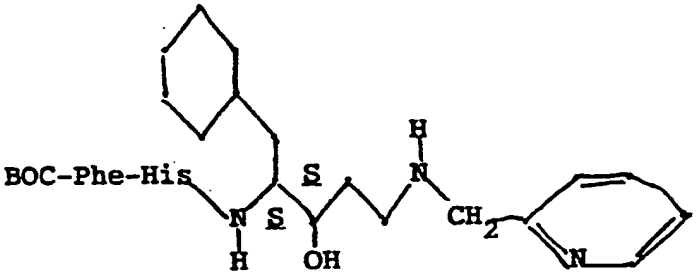

BOC-Phe-His
H
N
S
N
S
CH2
H
OH

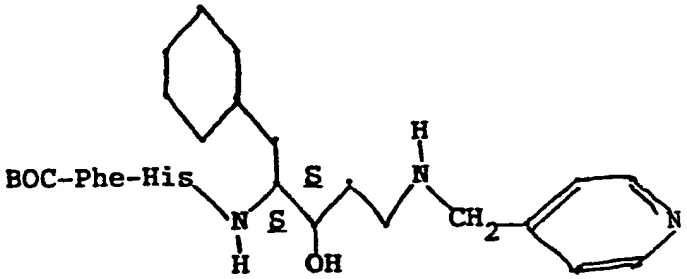

BOC-Phe-His
H
N
S
N
S
CH2CH(CH3)-CH2CH3
H
OH

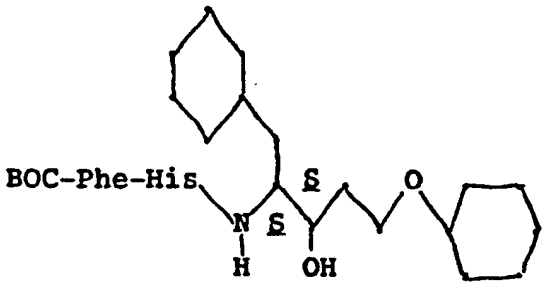

BOC-Phe-His
H
N
S
N
S
(CH2)2-CH(CH3)2
H
OH

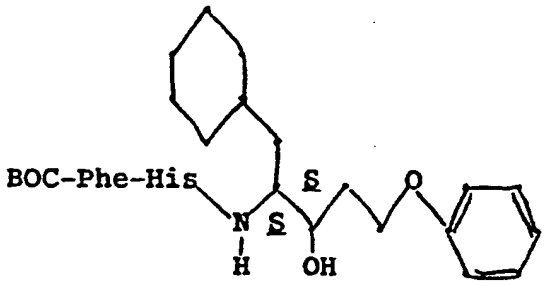

BOC-Phe-His
H
N
(CH2)3NH2
N
S
S
H
OH

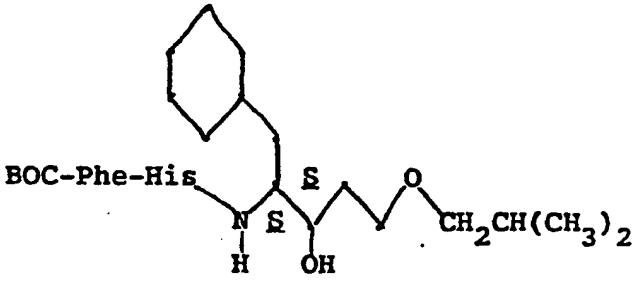

BOC-Phe-His
H
N
(CH2)4NH2
N
S
S
H
OH

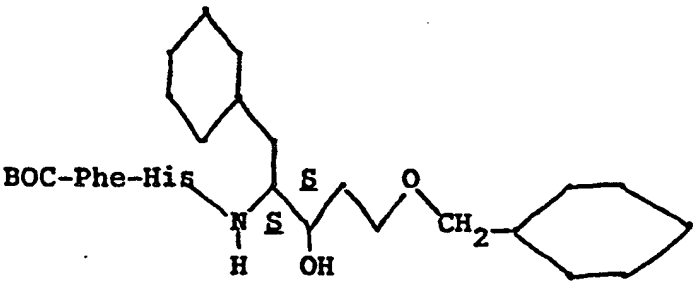

BOC-Phe-His
H
N
CH2
N
N
S
S
H
OH

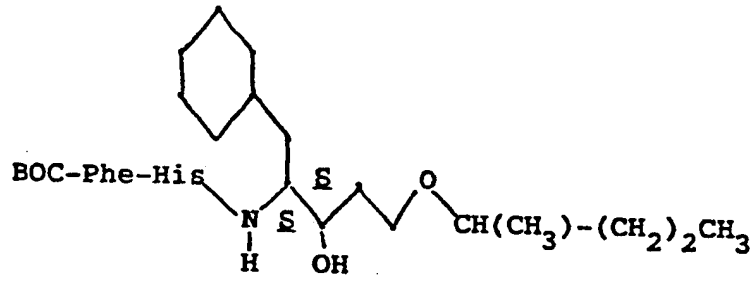

BOC-Phe-His
H
N
CH2
N
N
S
S
H
OH

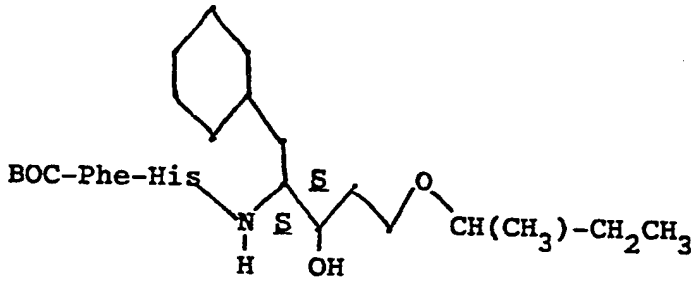

BOC-Phe-His
S
O
N
S
H
OH

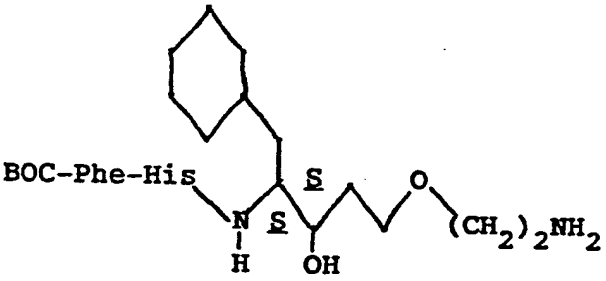

BOC-Phe-His
S
O
N
S
H
OH

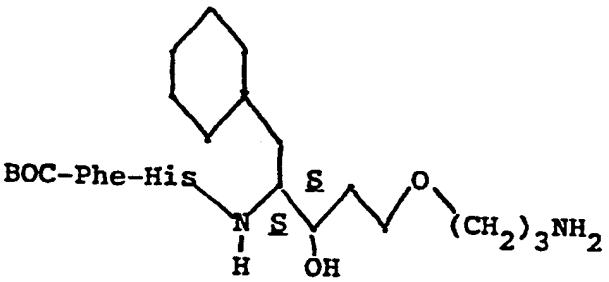

BOC-Phe-His
S
O
CH2CH(CH3)2
N
S
H
OH

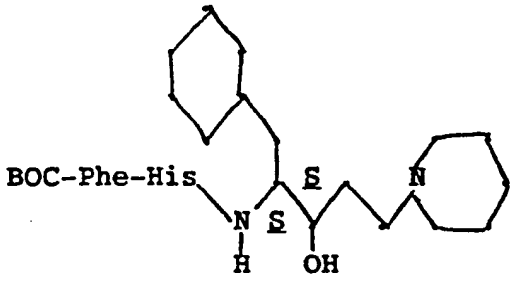

BOC-Phe-His
S
O
CH2
N
S
H
OH

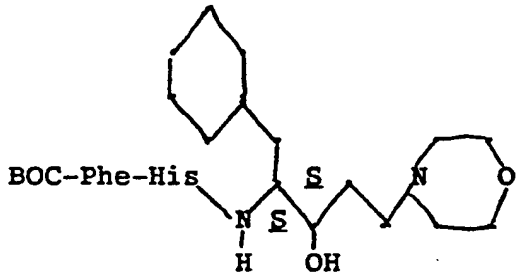

BOC-Phe-His
S
S
O
N
H
OH
CH(CH3)-(CH2)2CH3

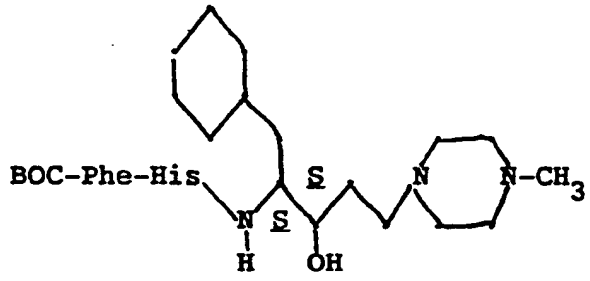

BOC-Phe-His
S
S
O
N
H
OH
CH(CH3)-CH2CH3

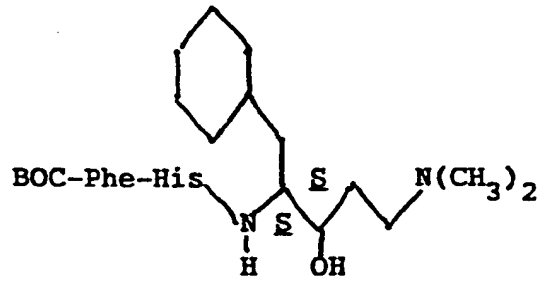

BOC-Phe-His
S
S
O
N
H
OH
(CH2)2NH2

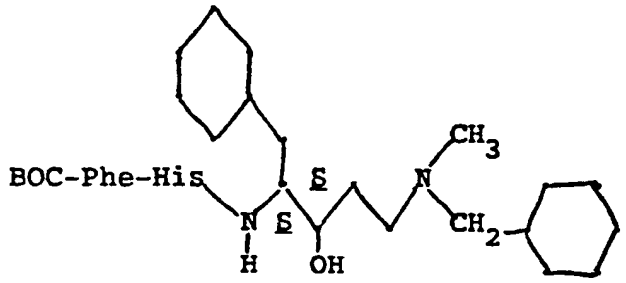

BOC-Phe-His
S
S
O
N
H
OH
(CH2)3NH2

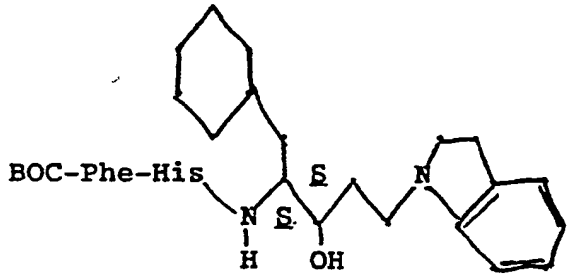

BOC-Phe-His
S
N
N
S
H
OH

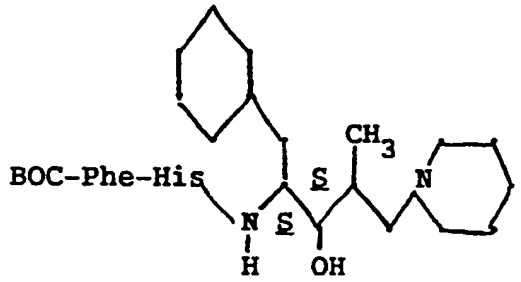

BOC-Phe-His
S
N
O
N
S
H
OH

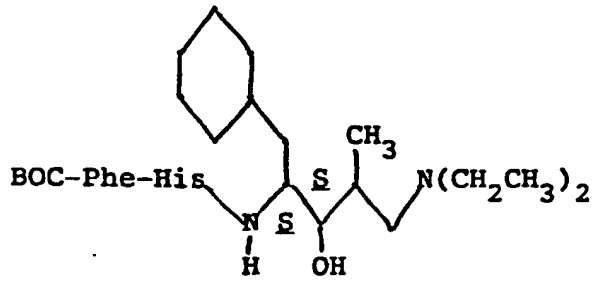

BOC-Phe-His
S
N
N-CH3
N
S
H
OH

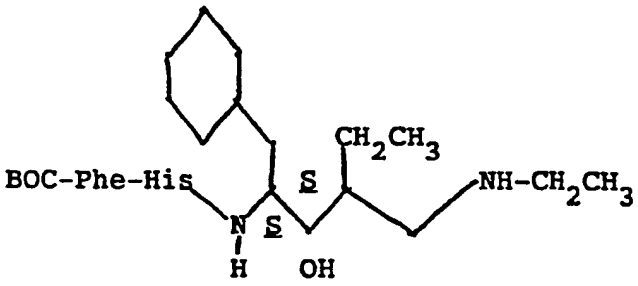

BOC-Phe-His
S
N(CH3)2
N
S
H
OH

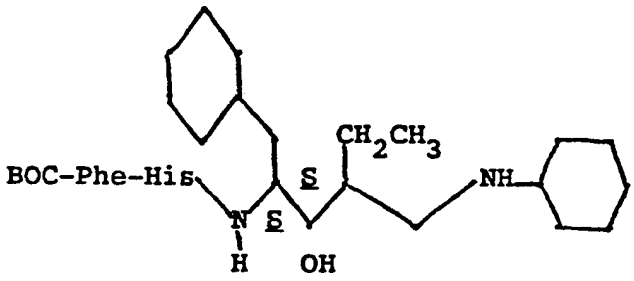

BOC-Phe-His
S
N
CH3
CH2
N
S
H
OH

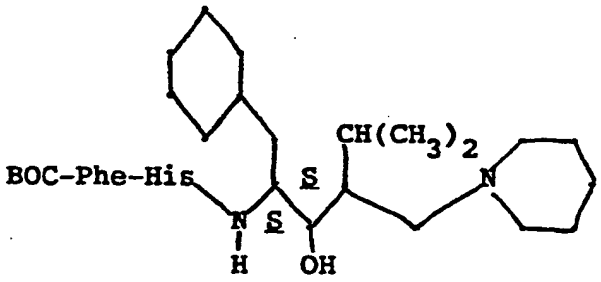

BOC-Phe-His
S
N
N
S
H
OH

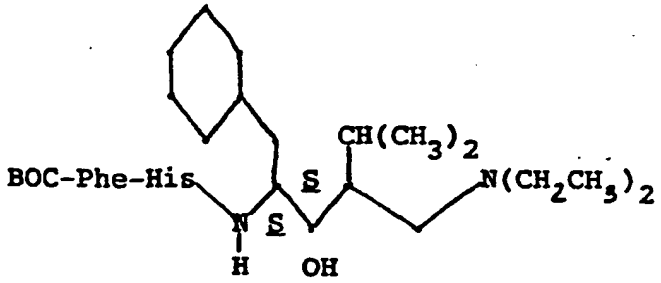

BOC-Phe-His
CH3
S
N
N
S
H
OH

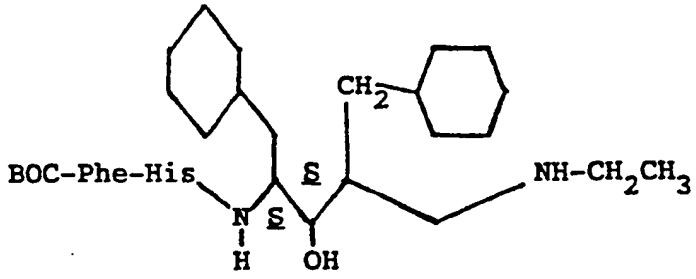

BOC-Phe-His
CH3
S
N(CH2CH3)2
N
S
H
OH

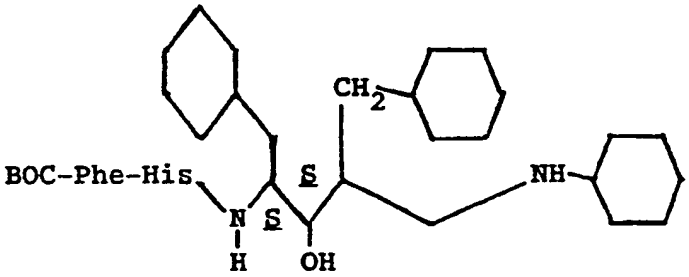

BOC-Phe-His
S
CH2CH3
NH-CH2CH3
N
S
H
OH

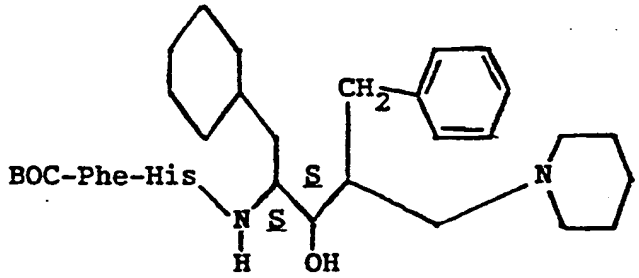

BOC-Phe-His
S
CH2CH3
NH
N
S
H
OH

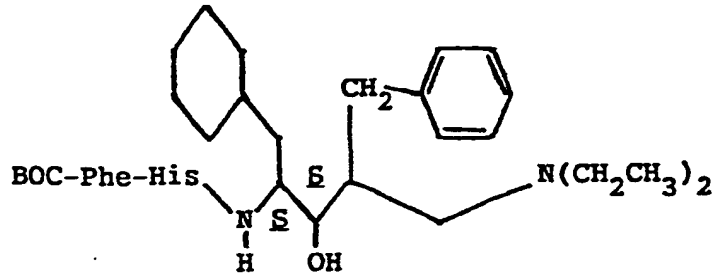

BOC-Phe-His
S
CH(CH3)2
N
N
S
H
OH

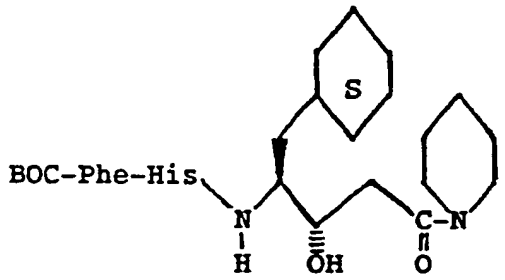

BOC-Phe-His
S
CH(CH3)2
N(CH2CH3)2
N
S
H
OH

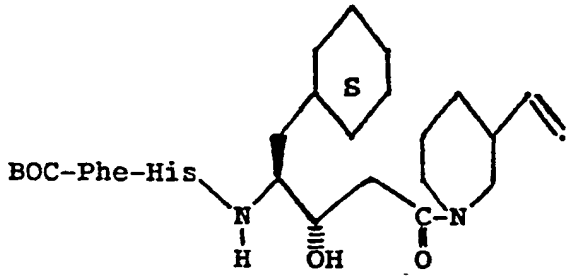

BOC-Phe-His
S
N
S
H
OH
CH2
NH-CH2CH3

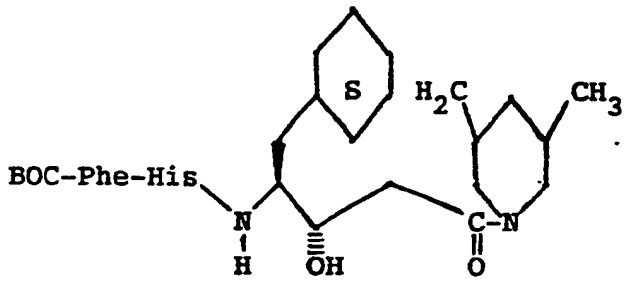

BOC-Phe-His
S
N
S
H
OH
CH2
NH

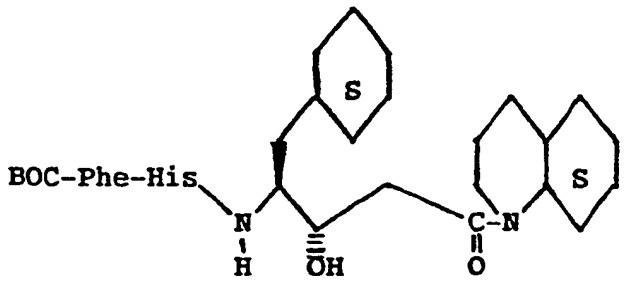

BOC-Phe-His
S
N
S
H
OH
CH2
N

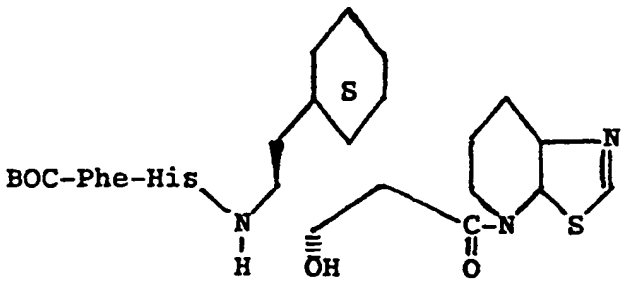

BOC-Phe-His
S
N
S
H
OH
CH2
N(CH2CH3)2

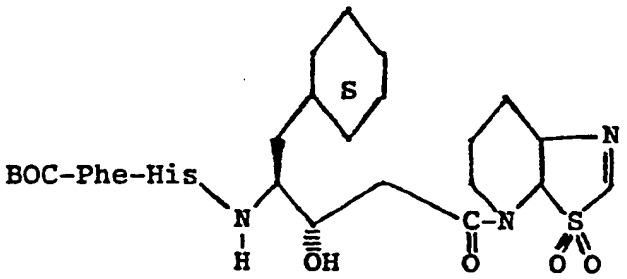

BOC-Phe-His
S
N
H
OH
C-N
O

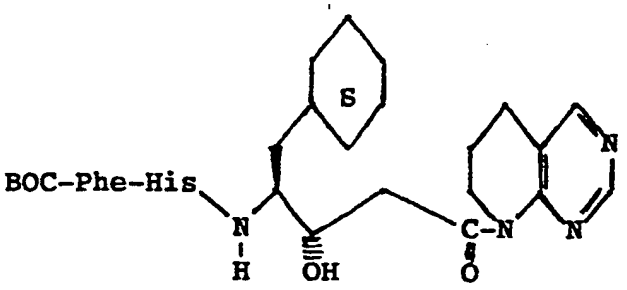

BOC-Phe-His
S
N
H
OH
C-N
O

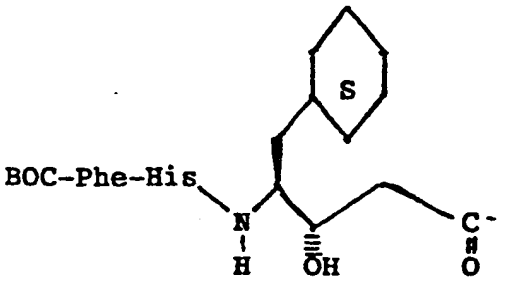

BOC-Phe-His
S
H2C
CH3
N
H
OH
C-N
O

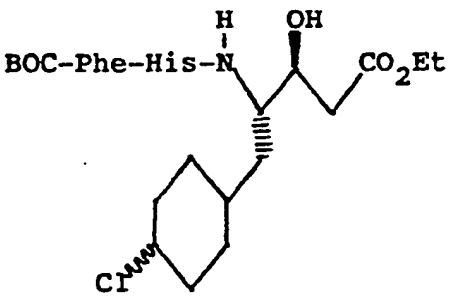

BOC-Phe-His
S
S
N
H
OH
C-N
O

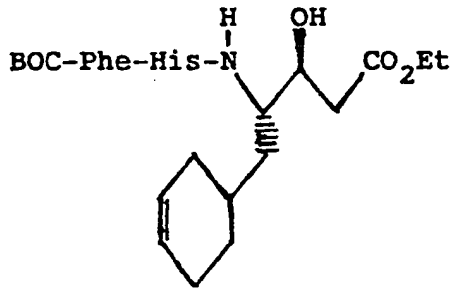

BOC-Phe-His
S
N
H
OH
C-N
O
N
S

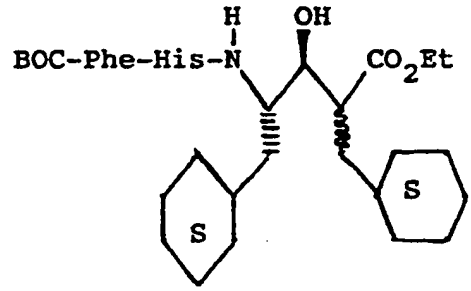

BOC-Phe-His
S
N
H
OH
C-N
O
N
S
O
O

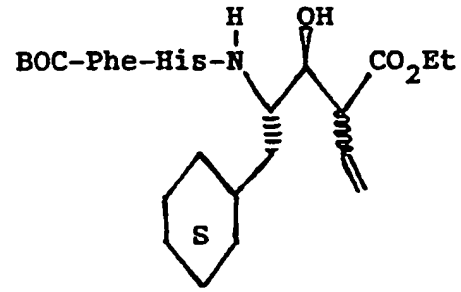

BOC-Phe-His
S
N
H
OH
C-N
O
N
N

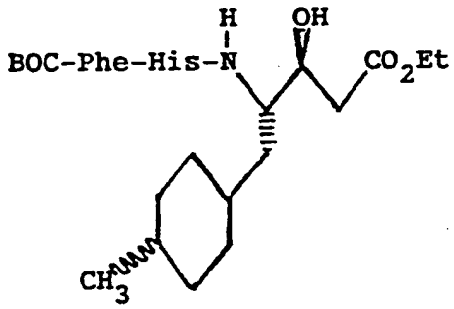

BOC-Phe-His
S
N
H
OH
C
O

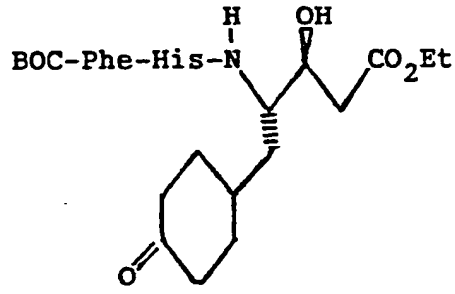

H
OH
BOC-Phe-His-N
CO2Et
Cl

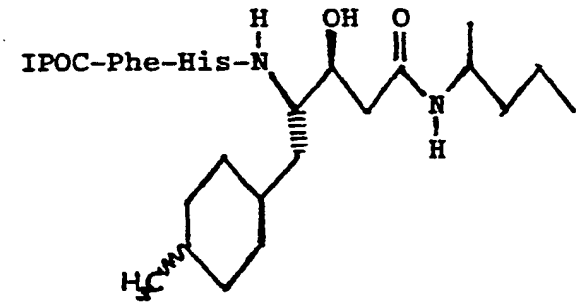

H
OH
BOC-Phe-His-N
CO2Et

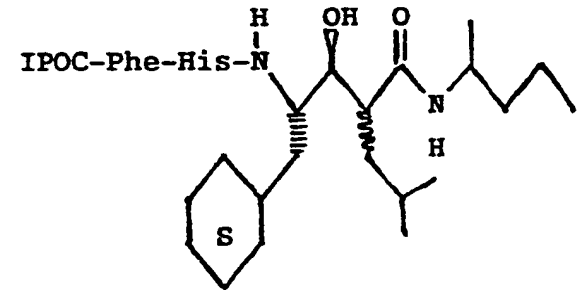

H
OH
BOC-Phe-His-N
CO2Et
S
S

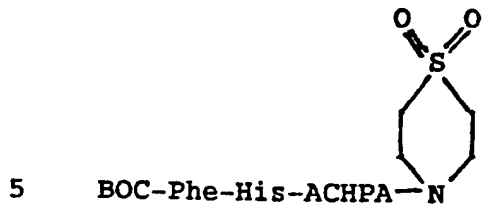

H
OH
BOC-Phe-His-N
CO2Et
S

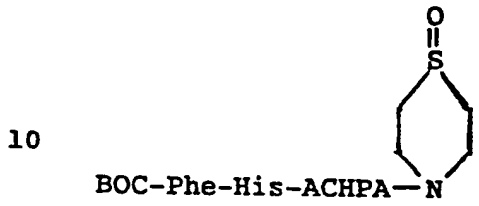

H
OH
BOC-Phe-His-N
CO2Et
CH3

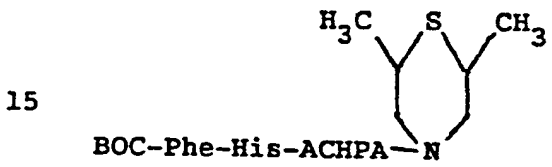

H
OH
BOC-Phe-His-N
CO2Et
O

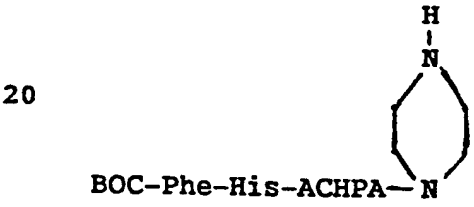

H
OH
O
IPOC-Phe-His-N
N
H
H3C

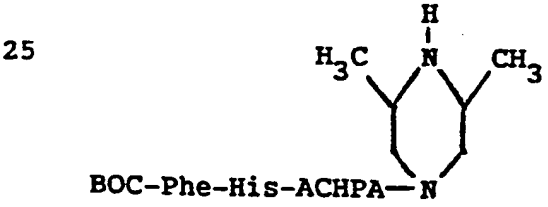

H
OH
O
IPOC-Phe-His-N
N
H
S

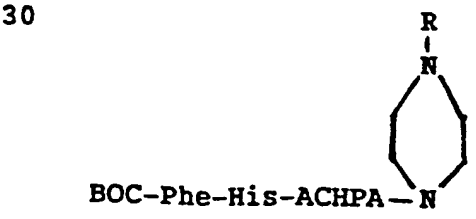




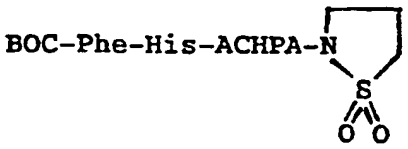


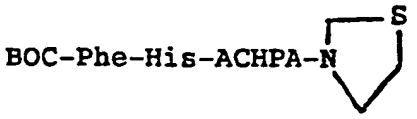


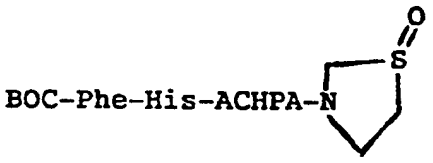


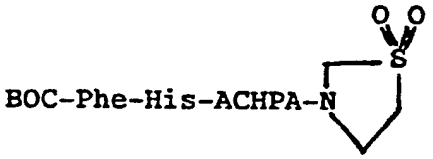


wher R=alkyl, aryl, $-CO_2H$, $-CH_2OH$

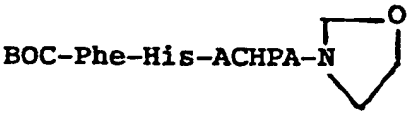

BOC-Phe-His-ACHPA-N
S
O O

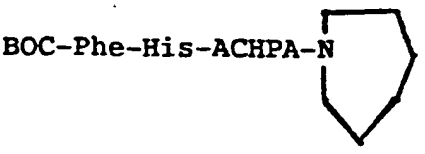

BOC-Phe-His-ACHPA-N
S


BOC-Phe-His-ACHPA-N
O
S

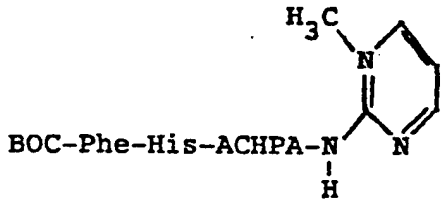

BOC-Phe-His-ACHPA-N
O O
S


BOC-Phe-His-ACHPA-N
N-CH3

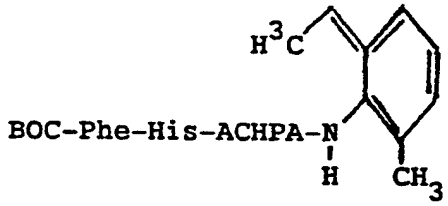

BOC-Phe-His-ACHPA-N
O


BOC-Phe-His-ACHPA-N

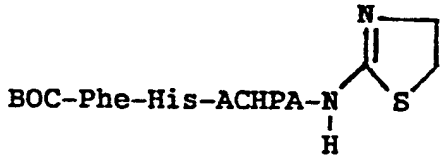
BOC-Phe-His-ACHPA-



BOC-Phe-His-ACHPA-



BOC-Phe-His-ACHPA-



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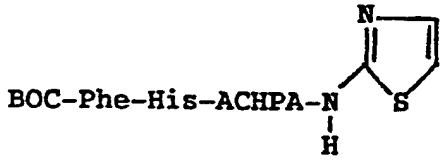

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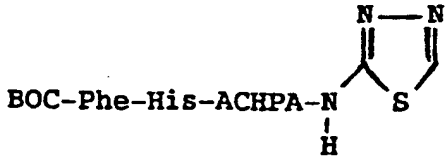

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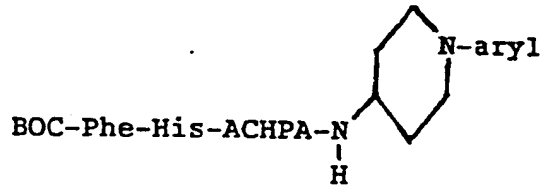
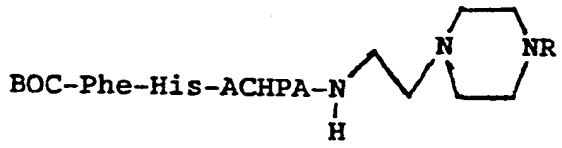
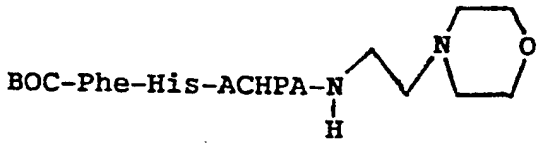
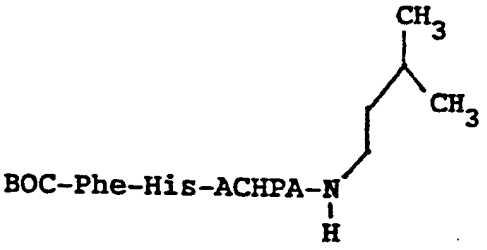
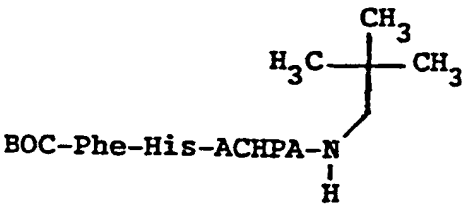


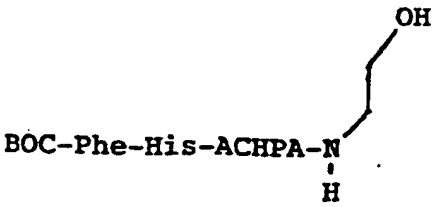


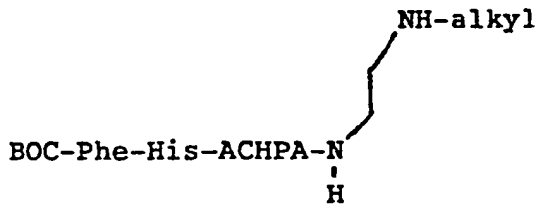
where R=H, alkyl

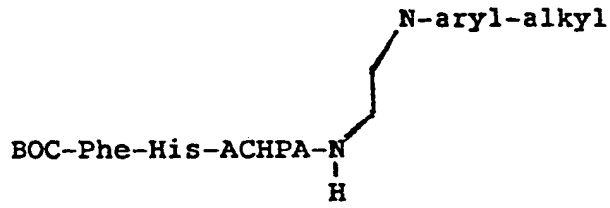









NH-alkyl
BOC-Phe-His-ACHPA-N
H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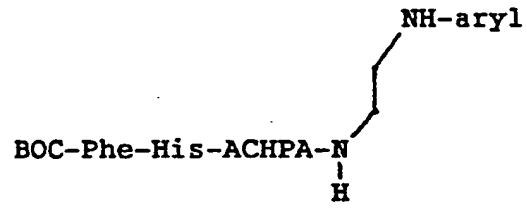
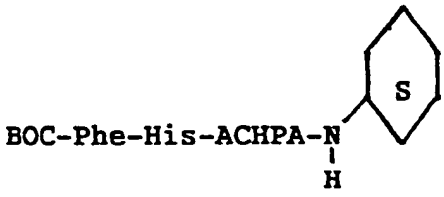
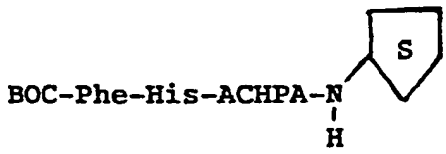
BOC-Phe-His-ACHPA-N
H


NH-aryl
BOC-Phe-His-ACHPA-N
H


S
BOC-Phe-His-ACHPA-N
H


S
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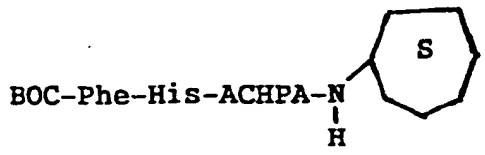
H


S
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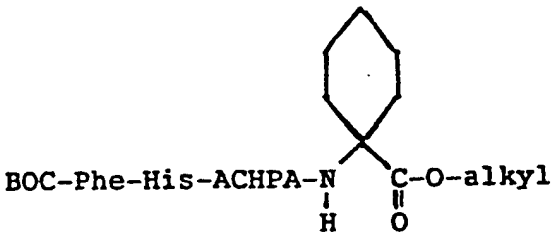
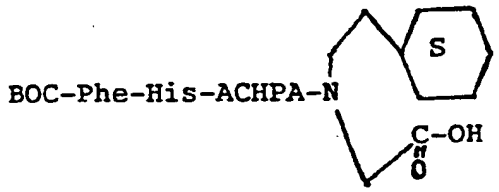

BOC-Phe-His-ACHPA-N
H
C-O-alkyl
O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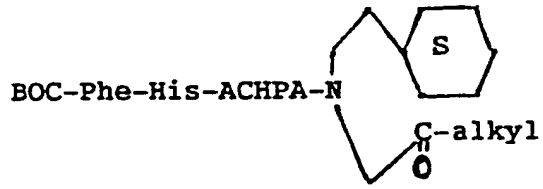
C-OH
O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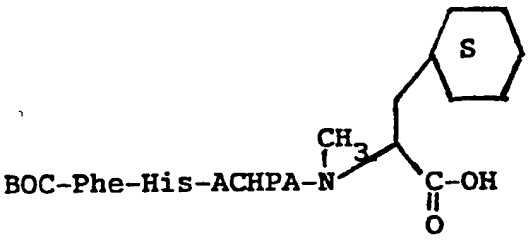
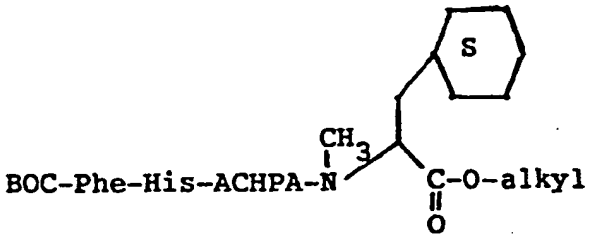
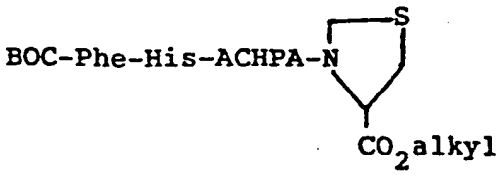
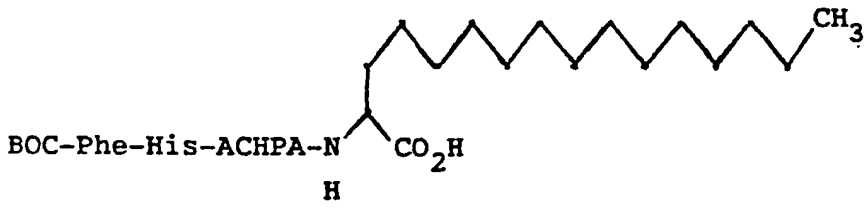
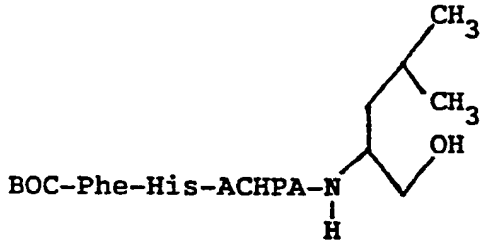
C-alkyl
O

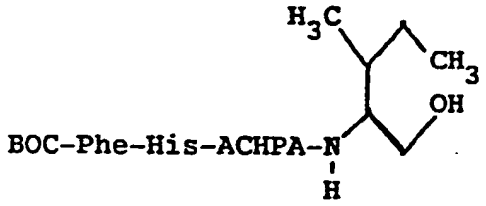

BOC-Phe-His-ACHPA-N
CH3
S
C-OH
O

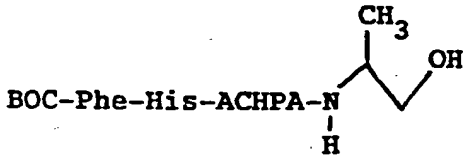

BOC-Phe-His-ACHPA-N
CH3
S
C-O-alkyl
O











BOC-Phe-His-ACHPA-$C_6H_{12}O_5N$

BOC-Phe-His-ACHPA-$C_5H_{10}O_4N$

EtO_2C-Phe-His-ACHPA-NH-[(2S)-methyl]butyl
2(S)-hydroxy-3-phenylpropionyl-His-ACHPA-NH-[92S)-methyl]butyl
S-benzylthioacetyl-His-Achpa-NH-[(2S)-methyl]butyl
Dibenzylacety-His-ACHPA-NH-[(2S0-methyl]butyl
Bis-(naphtylmethyl)acetyl-His-ACHPA-NH-[(2S)-methyl]butyl
Bis-(p-hydrocyphenylmethyl)acetyl-His-ACHPA-NH-[(2S)-methyl]butyl
2-Phenylamino-3-phenylpropionyl-His-ACHPA-NH-[(2S)-methyl]butyl
2-Phenyloxy-3-phenylpropionyl-His-ACHPA-NH-[(2S)-methyl-butyl[(2S)-me
2-Phenylthio-3-phenylpropionyl-His-ACHPA-NH-[(2S)-methyl]butyl
1,3-Diphenylpropyloxycarbon-His-ACHPA-NH-[(2S)-methyl]butyl
2-(1,3-diphenyl)propyloxycarbonyl-His-ACHPA-NH-[(2S)-methyl]butyl
2-Phenylthio-3-(1-naphthyl)propionyl-His-ACHPA-NH-[(2S)-methyl]butyl
[2-benzyl-2(3,4-dihydroxy)benzyl]acetyl-His-ACHPA-NH-[(2S)-methyl]butyl
[2-benzyl-2-(4-isopropyloxy)benzyl]acetyl-His-ACHPA-NH-[(2S)-methyl]butyl
BOC-Phe-His-[5-amino-66-cyclohexyl-4-hydroxy-2-isopropyl]hexanoyl 2(S)-aminobutane
BOC-Phe-His-[5-amino-6-cyclohexyl-4-hydroxy-2-isobutyl]hexanoyl 2(S)-aminobutane
BOC-Phe-His-[5-amino-2-benzyl-6-cyclohexyl-4-hydroxy]hexanoyl 2(S)-aminobutane

5. A pharmaceutical composition for treating renin-associated hyperaldosteronism, comprising a pharmaceutical carrier and a therapeutically effective amount of a peptide according to Claim 1.

6. A peptide of the formula:

$$A^{\circ}\text{-}B^{\circ}\text{-}D^{\circ}\text{-}E^{\circ}\text{-}G^{\circ} \quad (I^{\circ})$$

wherein:

A° is hydrogen, or R°_a-, R°_bCO or $R^{\circ}_bSO_2$- where R°_a and R°_b are alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heterocyclic, aryloxy alkyl, heterocyclic oxy alkyl, aryl alkyl, heterocyclic alkyl, heterocyclic oxyalkyl, and R°_a and R°_b may be

$R^{3'}$ is OH, NH_2, $NHR^{3'}_a$, $NR^{3'}_aN^{3'}_b$, $OR^{3'}_c$

where $R^{3'}_a$, $R^{3'}_b$, and $R^{3'}_c$ are separately alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkyl alkyl, aryl, aryl alkyl, heterocyclic, heterocyclic alkyl, each of which may be substituted with up to three members selected from amino, alkyl amino, dialkyl amino, trialkyl ammonium, hydroxy, alkoxy, aryloxy, aryl alkoxyl, or halo.

$R^{3'}_c$ may also be $R^{3'}_d$-CO-V'-$CR^{3'}_eR^{3'}_f$ wherein $R^{3'}_d$ is alkyl or aryl; $R^{3'}_e$ and $R^{3'}_f$ are hydrogen or alkyl; V' is -O- or -NH-.

$R^{4'}$ is hydrogen, alkyl, alkenyl, alkynyl, aryl, arylalkyl, each of which may be substituted with up to three members selected from amino, alkyl amino, dialkyl amino, trialkyl ammonium, hydroxy, alkoxy, halo or alkyl groups. $R^{4'}$ may also be $R^{4'}_a$-CO-V'-$CR^{4'}_bR^{4'}_c$ wherein $R^{4'}_a$ is alkyl, alkenyl or alkynyl, or aryl; $R^{4'}_b$ and $R^{4'}_c$ are hydrogen, alkyl, alkenyl, or alkynyl; V' is -O- or -NH-.

$R^{5'}$ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, cycloalkyl alkyl, aryl alkyl, heterocyclic, heterocyclic alkyl, aryloxy alkyl, heterocyclic oxy alkyl, heterocyclic oxy, each of which may be substituted with up to thre members s lected from amino, alkyl amino, dialkyl amino, trialkyl ammonium,

8. A peptide of Claim 6 wherein $R^{1'}$ is $-CH_2$-cyclohexyl.

9. A peptide of Claim 8 wherein $R^{6'}$ is H, and n' is 1.

10. A peptide of Claim 9 wherein $R^{2'}$ is $-CH(CH_3)_2$, $-CH_2-CH(CH_3)_2$, $-CH_2$-cyclohexyl and $R^{4'}$ is H.

11. A peptide of Claim 9 wherein $R^{6'}$ is H, $R^{2'}$ is H, n is 0, and $R^{4'}$ is H.

12. A compound of Claim 6 selected from

[N-(N-(N-carbobenzoxy-2-amino-3-(1-naphthyl)-propionyl)histidyl)-1-amino-2-cyclohexylethyl] 2-carboxy-4-methylpentylphosphinic acid;

[N-(N-(N-carbobenzoxy-2-amino-3-(1-naphthyl)-propionyl)histidyl)-1-amino-2-cyclohexylethyl] 2-carboxy-3-methylbutylphosphinic acid;

[N-(N-(N-t-butoxycarbonyl-2-amino-3-(1-naphthyl)-propionyl)histidyl)-1-amino-2-cyclohexylethyl] 2-carboxy-3-methylbutylphosphinic acid;

[N-(N-(N-carbobenzoxy-2-amino-3-(1-naphthyl)-propionyl)histidyl)-1-amino-2-cyclohexylethyl] 2-carbomethoxy-4-methylpentylphosphinic acid;

[N-(N-(N-carbobenzoxy-2-amino-3-(1-naphthyl)-propionyl)histidyl)-1-amino-2-cyclohexylethyl] 2-carboxamido-4-methylpentylphosphinic acid;

[N-(N-(N-t-butoxycarbonyl-2-amino-3-(1-naphthyl)-propionyl)histidyl)-1-amino-2-cyclohexylethyl] 2-(N-benzyl)carboxamido-3-methylbutylphosphinic acid;

Methyl [N-(N-(N-carbobenz xy-2-amino-3-(1-naphthyl)propionyl)histidyl)-1-amino-2-cyclohexyl-ethyl]carbomethoxymethylphosphinate; and

Ethyl [N-(N-3-phenylpropionyl-phenylalanyl)-1-amino-2-cyclohexylethyl] 2-carbomethoxy-3-methyl-butylphosphinate.

13. A pharmaceutical composition for treating hypertension or congestive heart failure containing a compound of Claim 6.

14. A pharmaceutical composition for treating hypertension containing a compound of Claim 1 or 6 and one or more antihypertensive agents selected from the group consisting essentially of:

Diuretics: acetazolamide; amiloride; bendroflumethiazide; benzthiazide; bumetanide; chlorothiazide; chlorthalidone; cyclothiazide; ethacrynic acid; furosemide; hydrochlorothiazide; hydroflumethiazide; indacrinone (racemic mixture, or as either the (+) or (-) enantiomer alone, or a manipulated ratio, e.g., 9:1 of said enantiomers, respectively); metolazone; methyclothiazide; muzolimine; polythiazide; quinethazone; sodium ethacrynate; sodium nitroprusside; spironolactone; ticrynafen; triamterene; trichlormethiazide;

((±)-3'-acetyl-4'-(2-hydroxy-3-isopropylaminopropoxy)-acetanilide HCl) (diacetolol);

(methyl-4-[2-hydroxy-3-[(1-methylethyl)aminopropoxy]]-benzenepropanoate HCl) (esmolol);

(erythro-DL-1-(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol);

(1-(tert.butylamino)-3-[O-(2-propynyloxy)phenoxy]-2-propanol (pargolol);

(1-(tert.butylamino)-3-[o-(6-hydrazino-3-pyridazinyl)-phenoxy]-2-propanol diHCl) (prizidilol);

((-)-2-hydroxy-5-[(R)-1-hydroxy-2-[(R)-(1-methyl-3-phenylpropyl)amino]ethyl]benzamide);

(4-hydroxy-9-[2-hydroxy-3-(isopropylamino)-propoxy]-7-methyl-5H-furo[3,2-g][1]-benzopyran-5-one) (iprocrolol);

((-)-5-(tert.butylamino)-2-hydroxypropoxy]-3,4-dihydro-1-(2H)-naphthalenone HCl) (levobunolol);

(4-(2-hydroxy-3-isopropylamino-propoxy)-1,2-benziso-thiazole HCl);

(4-[3-(tert.butylamino)-2-hydroxypropoxy]-N-methyliso-carbostyril HCl);

((±)-N-2-[4-(2-hydroxy-3-isopropyl aminopropoxy)-phenyl]ethyl-N'-isopropylurea) (pafenolol);

(3-[[(2-trifluoroacetamido)ethyl]amino]-1-phenoxy-propan-2-ol);

(N-(3-(o-chlorophenoxy)-2-hydroxypropyl)-N'-(4'-chloro-2,3-dihydro-3-oxo-5-pyridazinyl)ethylenediamine);

((±)-N-[3-acetyl-4-[2-hydroxy-3-[(1-methylethyl)amino]-propoxy]phenyl]butanamide) (acebutolol);

((±)-4'-[3-(tert-butylamino)-2-hydroxypropoxy]spiro-[cyclohexane-1,2'-indan]-1'-one) (spirendolol);

(7-[3-[[2-hydroxy-3-[(2-methylindol-4-yl)oxy]propyl]-amino]butyl]thiophylline) (teoprolol);

(1-(4-(2-(4-fluorophenyloxy)ethoxy)phenoxy)-3-isopropylamino-2-propanol HCl);

((-)-p-[3-[(3,4-dimethoxyphenethyl)amino]-2-hydroxypropoxy]-ß-methylcinnamonitrile) (pacrinolol);

((±)-2-(3'-tert.butylamino-2'-hydroxypropylthio)-4-(5'-carbamoyl-2'-thienyl)thiazole HCl) (arotinolol);

((±)-1-[p-[2-(cyclopropylmethoxy)ethoxy]phenoxy]-3-(isopropylamino)-2-propanol) (cicloprolol);

((±)-1-[(3-chloro-2-methylindol-4-yl)oxy]-3-[(2-phenoxyethyl)amino]-2-propanol) (indopanolol);

((±)-6-[[2-[[3-(p-butoxyphenoxy)-2-hydroxypropyl]-amino]ethyl]amino]-1,3-dimethyluracil) (pirepolol);

(4-(cyclohexylamino)-1-(1-naphtholenyloxy)-2-butanol);

(1-phenyl-3-[2-[3-(2-cyanophenoxy)-2-hydroxypropyl]-aminoethyl]hydantoin HCl);

(3,4-dihydro-8-(2-hydroxy-3-isopropylaminopropoxy)-3-nitroxy-2H-1-benzopyran) (nipradolol);

<u>α and ß-Adrenergic Blocking Agents</u>:

((±)-1-tert-butylamino)-3-[o-[2-(3-methyl-5-isoxazolyl)vinyl]phenoxy]-2-propanol) (isoxaprolol);

(1-isopropylamino-3-(4-(2-nitroxyethoxy)phenoxy)-2-propanol HCl);

(4-hydroxy-α-[[3-(4-methoxyphenyl)-1-methylpropyl]-aminomethyl]-3-(methylsulfinyl)-benzmethanol HCl) (sulfinalol);

(5-[1-hydroxy-2-[[2-(o-methoxyphenoxy)ethyl]amino]-ethyl]-2-methylbenzenesulfonamide HCl);

(5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl]-salicylamide HCl) (labetalol);

(2-(N-benzyl-N-methylamino)ethylmethyl-2,6-dimethyl-4-(m-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate HCl) (nicardipine);

(N-(3,4-dimethoxyphenethyl)-2-(3,4-dimethoxyphenyl)-N-methyl-m-dithiane-2-propylamine-1,1,3,3-tetraoxide) (tiapamil);

(5,6-dimethoxy-2-(3-[(α-(3,4-dimethoxy)phenylethyl)-methylamino]propyl)phthalimidine) (falipamil);

(ß-[(2-methylpropoxy)methyl]-N-phenyl-N-phenylmethyl-1-pyrrolidineethanamine HCl monohydrate) (bepridil);

((+)-cis-3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4-(5H)-one) (diltiazem);

((E)-1-[bis-(p-fluorophenyl)methyl]-4-cinnamylpiperazine di HCl) (flunarizine);

(5-[(3,4-dimethoxyphenethyl)methylamino]-2-isopropyl-2-(3,4,5-trimethoxyphenyl)valeronitrile (gallopamil);

(ethylmethyl(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate (felodipine);

(isopropyl-2-methoxyethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinecarboxylate) (nimodipine);

(3-ethyl-5-methyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylate) (nitrendipine);

<u>Angiotensin I Converting Enzyme Inhibitors</u>:

1-(3-mercapto-2-methyl-1-oxopropyl)-L-proline (captopril);

(1-(4-ethoxycarbonyl-2,4(R,R)-dimethylbutanoyl)-indoline-2(S)-carboxylic acid);

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E-Mail: nerac@nerac.com

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Ms. Carrie Barclay
Order Number: 01450699
Patent Number: P0212903
2 of 10

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Ms. Carrie Barclay
Information Assistant
Elan Pharmaceutical
800 Gateway Boulevard
South San Francisco, CA 94080